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FREDERICK CHARLES NEWCOMBE
1858-1927

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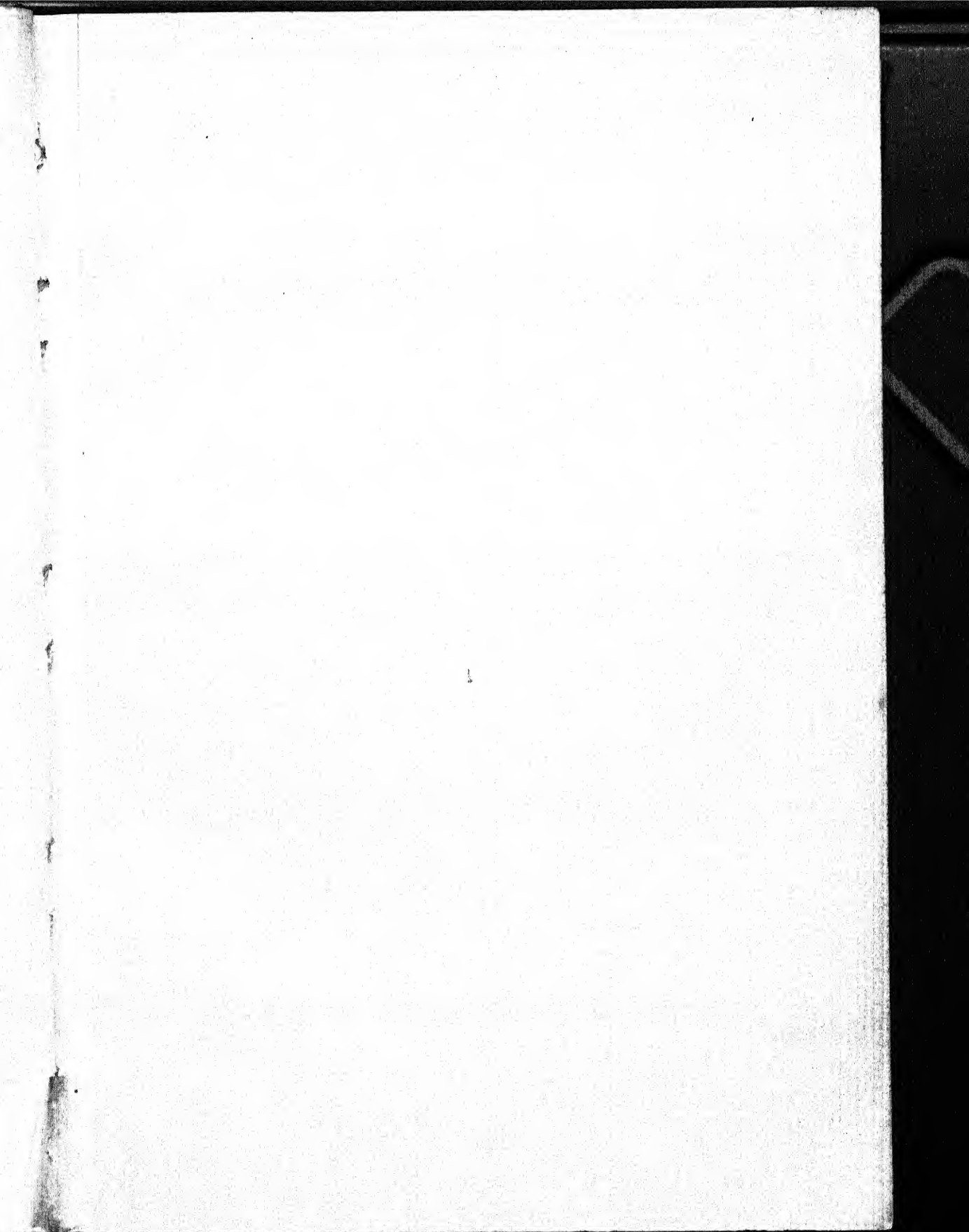
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ERRATA, VOLUME XV

Page 141, 2d and 3d lines of 2d paragraph. For <i>Liquidamber</i> , read <i>Liquidambar</i> .
Page 200, 17th line from bottom. For <i>betuminosum</i> , read <i>bituminosum</i> .
Page 429, 16th line from top. For <i>Phorothamnus</i> , read <i>Psorothamnus</i> .
Page 491, 3d line from top. For <i>stagnales</i> , read <i>stagnalis</i> .
Page 555, in the subtitle under "Discussion". <i>Pressure</i> should be <i>Presence</i> .
Page 586, 16th line from bottom. For <i>glyciphylloides</i> , read <i>glyciphylloides</i> .





H. C. Newcombe

AMERICAN JOURNAL OF BOTANY

VOL. XV

JANUARY, 1928

No. 1

FREDERICK CHARLES NEWCOMBE, 1858-1927¹

J. B. POLLOCK AND H. H. BARTLETT

In the death of Doctor Frederick Charles Newcombe at Honolulu on October 1, 1927, the Botanical Society of America has lost one of its most loyal members. His services to the Society, to the University of Michigan, and to the science of botany are hard to summarize in a brief memorial. Throughout his life he was intensely devoted to developing higher professional standards among university men. At a time when such a policy was distinctly an unpopular one, he strongly advocated the principle that research as well as teaching should be an essential part of the duty of members of a university faculty. Nowadays, when university administrations take this for granted, it is difficult to realize that quite a different attitude has until recently prevailed in America. In the early history of the Research Club at the University of Michigan, Professor Newcombe was closely identified with the efforts of this Club to bring about a general acceptance of productive scholarship as a criterion of academic advancement in the faculty.

Professor Newcombe participated in the work of all organizations that sought to promote solidarity among botanists, or that led younger men into more intimate contacts with the older members of the profession. He founded the Botanical Journal Club at the University of Michigan, the first of a now large group of departmental organizations that have as an object the induction of able students into research through acquaintance with literature, and through forming personal contacts with the faculty. The Club which he founded has been the model for many similar organizations in other universities and colleges. His interest in the Michigan Academy of Science was always keen. He was secretary at its organization in 1894, and was president in 1903-4. There was never a year when he did not take a personal interest in the arrangement of the Academy program, to the great advantage of successive chairmen of the botanical section. During the time that the society known as the Botanists of the Central

¹ Prepared at the request of the Editorial Board. Frontispiece is from a painting by Leon Makielski, presented to the University of Michigan by former students and colleagues in 1923, and here reproduced by courtesy of the University.

States was actively functioning, he took much interest in its affairs and was its president in 1906. This society waned after the inauguration of a more liberal membership policy on the part of the Botanical Society of America, but during the period of its activity it filled an important place. Professor Newcombe was always active in the affairs of the American Association for the Advancement of Science and of the Botanical Society of America. He seldom missed the annual meetings, and was the secretary of section G (Botany) of the former in 1897, and the president of the latter in 1917. He was secretary of the section of Plant Physiology at the International Congress of Arts and Science at St. Louis in 1904.

It was due to his repeated recommendations through a period of years and much urging of botanists through personal correspondence that the Botanical Society of America founded its research journal, the *AMERICAN JOURNAL OF BOTANY*, in 1914. He was the editor-in-chief of this Journal until 1918. It is now believed to be more widely distributed than any other technical botanical journal in the world. To have been influential in founding it and to have edited it during the period when its reputation was being established constitute a great accomplishment for botany and his chief contribution to the Botanical Society of America. The large number of organizations which he helped to establish or support is evidence of his initiative and leadership.

After Professor Newcombe's retirement, as Professor Emeritus, in 1923, he removed to Hawaii and became the center of the botanical group in Honolulu. He was instrumental in founding there two active and useful organizations, the Hawaiian Botanical Society and the Hawaiian Academy of Science, and was the first president of each.

Although physically frail, Newcombe led a life of great activity. He not only helped to shape a University policy with regard to research but was also no less interested in educational policies. He was ever zealous in maintaining a close relation between the secondary schools and the University. He spared no pains to make the annual visit of the high school teachers to Ann Arbor, on the occasion of the meeting of the Michigan Schoolmaster's Club, pleasant and profitable, and was very impatient with any occasional tendency of his colleagues to shirk responsibilities on this occasion. Among his services to the University of Michigan may be mentioned his aid in the establishment of the original Botanical Garden (now the Nichols Arboretum of the Department of Landscape Design), through a gift of land which he was influential in securing from his friends Dr. Walter H. and Esther B. (Connor) Nichols; and later, the founding of the present Botanical Garden, in which he took an important part.

His influence will long be felt as a painstaking and conscientious teacher and as an uncompromising enemy of low ideals in investigation and scholarship. During his long career at the University of Michigan, from 1890 until 1923, he lived simply and unostentatiously, and only after his death

was it learned that he had left to the University the residue of his estate to found one or more fellowships in his own special field, plant physiology. His will provides for the foundation, and states that the fellows who benefit by it shall have the opportunity of a year of foreign study before taking the doctorate.

The clause of the will providing for the foundation is as follows:

"I give, devise and bequeath all the rest, residue and remainder of my estate both real and personal and wheresoever situate, unto the Board of Regents of the University of Michigan, Ann Arbor, Michigan, to have and to hold absolutely and in fee simple, but in trust nevertheless, and for the uses and purposes and with the powers hereinafter set forth, *viz*:

"(a) This foundation to be known as the F. C. and Susan Eastman Newcombe Fellowship in Plant Physiology.

"(b) The income of this foundation to be used for the purpose of employing and/or paying the expenses of one or more investigators in the subject of plant physiology.

"(c) It is my desire and I direct that the person or persons appointed to this fellowship shall have shown more than ordinary promise of distinguished achievement in science, and I request (although this is not mandatory) that the person or persons about to be appointed to this fellowship shall, at the expense of the Trust, spend at least one year in some foreign institution of learning, preferably in Germany or France."

Professor Newcombe was born at Flint, Michigan, May 8, 1858. He received his B.S. degree at the University of Michigan in 1890 and his Ph.D. under Pfeffer, at the University of Leipzig, in 1893. He was Instructor in Botany 1890-1892, Assistant Professor 1893-1897, Junior Professor 1897-1905, and Professor after 1905. He succeeded Professor Volney M. Spalding as Head of the Department of Botany in 1904. From 1880 to 1887 he was a teacher in the Michigan School for the Deaf. Although late in making a start in his chosen profession, he accomplished a respectable volume of excellent research and published on a variety of subjects, mostly concerned with the sensitive reactions of plants. He was married June 26, 1884, to Susan Eastman of Flint, Michigan. Both Mr. and Mrs. Newcombe were in poor health at the time of his retirement in 1923. During the last two years of Mrs. Newcombe's life the care and strain of her illness did much to hasten his own end. He died October 1, 1927, the day after his return to Honolulu from Michigan, where he had taken his wife's remains for burial. In his passing we have lost one of the dominating figures in botanical science in America.

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STUDIES ON THE GROWTH OF ROOT HAIRS IN SOLUTIONS

IV. THE PH-MOLAR-RATE RELATION FOR COLLARDS IN CALCIUM CHLORID¹

CLIFFORD H. FARR

(Received for publication January 6, 1927)

The preceding paper (III) included a study of the rate of elongation of root hairs of Georgia collards for a period of three hours beginning thirteen hours after immersion of the radicles in flowing solutions of calcium chlorid of varying molar concentrations. These solutions were adjusted to a hydrogen-ion concentration of approximately 7.9. There was also presented the results of the same type of study with different concentrations of calcium hydroxid, varying in hydrogen-ion concentration from neutrality to 11.9. It is obvious that the two graphs representing these data may be regarded as being at right angles to each other, the former along the molar scale and the latter along the pH scale, with the rate of elongation constituting the third dimension. It is now proposed to ascertain the rate of elongation in various hydrogen-ion concentrations at certain selected molar concentrations of this salt, and to construct a solid figure representing this triple relationship.

The hydrogen-ion concentrations were determined by the indicator method. The hydrogen electrode method was considered inexpedient in this case because of the difficulty of handling unbuffered solutions with that method, and because of the wide intervals (0.5 to 1 pH unit) between the solutions prepared, making delicate precision unnecessary. The indicators employed were as follows: Brom Phenol Blue, 3.0-4.6; Methyl Red, 4.4-6.0; Brom Cresol Purple, 5.4-7.0; Brom Thymol Blue, 6.0-7.6; Phenol Red, 6.6-8.2; Cresol Red, 7.2-8.8; Thymol Blue, 8.2-9.8; Phenolphthalein, 8.5-10.5; Alizarine Yellow R, 10.1-12.1; Methyl Blue, 10.0-13.0.

In the range from pH 5.3 to 8.0 the color of the solution after adding the dye was compared with that of a Sorensen phosphate buffer solution to which an equal quantity of the dye had been added. Outside of this range the color chart in Clark's book (142) was used as far as it went. Above 9.9 it was found very difficult to determine the hydrogen-ion concentration with a fair degree of accuracy. This portion of the pH scale is known to be very unstable, and the data obtained for this region are regarded as being for the most part of only relative significance.

The procedure for the preparation of solutions was as follows. Distilled water was obtained from either of two Precision copper stills at the Marine

¹ This paper is the fourth of a series of six appearing in successive issues of the Journal.

Biological Laboratory. It was first made approximately neutral and employed at a pH of about 6.9. To this was added enough of the stock solution of calcium chlorid, prepared as described in the first paper of this series, to bring the solution to the desired molar concentration. In case the hydrogen-ion concentration of this solution was found not to be that desired, either calcium hydroxid or hydrochloric acid was added to render the solution more alkaline or acid, respectively.

It was found that a mixture of the stock calcium chlorid solution with distilled water gave a solution with a pH value of more than 6.9. The hydrogen-ion concentrations of the unadjusted solutions were as follows: 0.008 *M*, 7.1; 0.020 *M*, 7.4; 0.028 *M*, 7.9; 0.060 *M*, 8.9; 0.120 *M*, 9.2. If these data were plotted with molar concentrations as ordinates and hydrogen-ion concentrations as abscissae, a sigmoid graph would be obtained. This is the typical graph for a titration curve of a weak acid and a strong base. Pure calcium chlorid in neutral distilled water should give a titration curve which is nearly a straight line. The explanation of this sigmoid graph, that is, of increasing alkalinity with concentration, is doubtless to be found in the presence of calcium oxid as an impurity in the salt, which, upon dissolving, forms calcium hydroxid. It was noted in the first paper of the series that the calcium chlorid used contained a trace of calcium oxid. It is also likely that heating the salt to 175° C., to drive off the water, resulted in decomposing some of the salt into hydrochloric acid and calcium oxid. The former being volatile at these high temperatures, the latter would be left, increasing the amount of that substance as an impurity in the salt. Since the solutions were adjusted, if necessary, by the addition of either calcium hydroxid or hydrochloric acid, the presence of calcium oxid in the dry salt used did not constitute a source of error in the experiments.

The addition of calcium hydroxid and of hydrochloric acid to the calcium chlorid solution increases the concentration of the calcium or of the chlorine ions, respectively, above that of the original molar concentration of the salt solution. The question may be raised as to whether, therefore, the readings for the more acid or more alkaline solutions are exactly comparable to those of unadjusted solutions. The amounts of calcium hydroxid required to raise the pH value of two liters of an 0.008 *M* calcium chlorid solution to 7.9, 8.9, 9.9, 10.9, and 11.9, respectively, are 1.8, 3.0, 5.5, 9.0, and 100.0 cc. of a saturated solution. The last-named, which is the largest amount used, contains enough calcium to raise the calcium concentration of an 0.008 molar calcium chlorid solution to that of a 0.009 *M* solution. Inasmuch as the next higher concentration studied with respect to root-hair elongation was 0.020 *M*, the alteration of the calcium content in the adjustment of the solutions to the desired pH value is negligible. A similar situation obtains as regards the concentration of chlorine ions in acidulation.

Another question which may arise is as to whether or not the hydrogen-ion concentration of the solution in the flask supplying the chamber on the

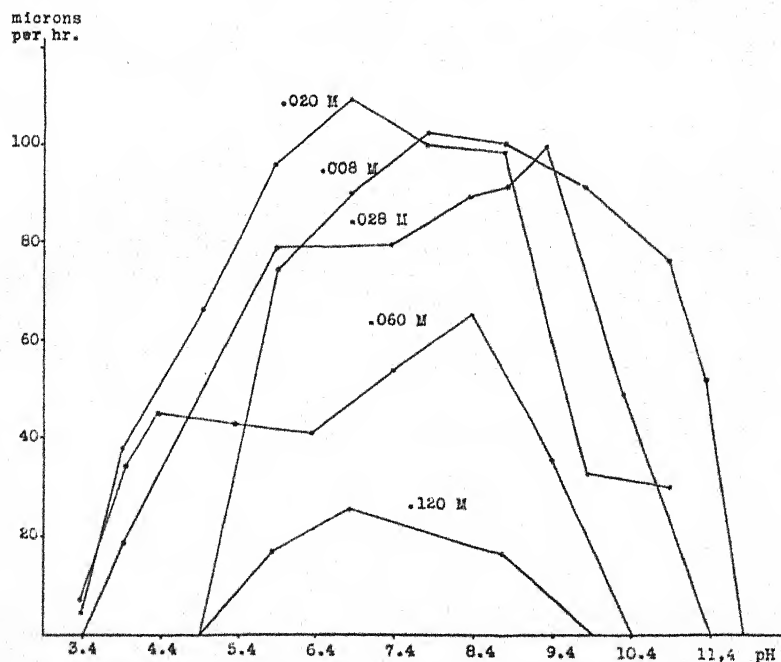
microscope remained reasonably constant during the period of the experiment. The hydrogen-ion concentration of the freshly mixed solution was tested by the indicator method just before the insertion of the flask into the apparatus. It was then aerated, as described before, with carbon-dioxid-free air, and allowed to flow out through the siphon without coming into contact with free air again. At the close of the experiment, that is, about 20 hours after insertion of the flask, the sample of solution remaining in it was tested with the indicators. In no case was a change of more than two or three tenths of one pH unit noted. This constancy of the hydrogen-ion concentration of an unbuffered solution is doubtless to be attributed to its freedom from carbon dioxid.

TABLE 8. *Root-hair Elongation in 0.008 M CaCl₂*

pH	Average Microns per Hour	Av.	Max.
3.9	42 hairs measured; no growth in any of them.	0	0
4.9	21 hairs measured; no growth in any of them.	0	0
5.9	74.0; 47.9; 39.9; 54.7; 44.8; 48.5; 71.5; 52.6	54.2	74.0
6.9	60.6; 55.0; 56.0; 52.9; 84.0; 53.8; 61.2; 65.3; 45.7; 82.1; 77.1; 59.1; 62.2; 73.7; 90.2	66.5	90.2
7.9	68.7; 97.3; 96.4; 101.1; 100.2; 87.4; 94.2; 88.9; 83.4; 99.2; 65.3; 102.6; 99.2; 76.1; 76.4; 77.7	88.4	102.6
8.9	84.2; 84.6; 70.6; 84.6; 79.9; 83.7; 69.7; 90.8; 88.0; 99.9; 77.7; 87.7; 40.8; 65.3; 65.3; 27.8	77.6	99.9
9.9	70.3; 87.1; 79.0; 81.8; 77.1; 91.1; 66.2; 87.4; 65.3; 80.9; 60.6; 64.1; 78.4; 79.9; 84.0	76.9	91.1
10.9	53.8; 49.8; 59.2; 52.9; 39.5; 42.0; 60.6; 55.3; 65.9; 67.5; 60.0; 67.8; 61.1; 76.1; 70.6; 57.5	58.7	76.1
11.4	47.3; 47.3; 47.3; 50.5; 45.1	47.5	50.4
11.9	11 hairs measured; no growth in any of them.	0	0

The measurements of all root hairs in 0.008 molar CaCl₂ solutions are included in table 8 except 10 hairs in 11.4 which did not grow at all. Had they been included, the average for this hydrogen-ion concentration would be 15.8 instead of 47.5. Text figure 5, based upon the maximum readings, shows only one maximum at this pH, namely, at 7.9. Text figure 6 for the average readings shows a slight depression between the two optima at 7.9 and 9.9, the former of which is considerably the greater. If the last reading in 8.9, which was of a hair which stopped growth after 80 minutes, were omitted, the graph would be approximately flat between 8.9 and 9.9.

From the data in table 9 there are omitted the records of the following root hairs, which did not grow at all: 25 in 3.4; 11 in 3.9; 4 in 11.4. There are also omitted the readings of two root hairs in 6.9 which stopped growing before the close of the three hours. Their averages were 55.3 and 52.9.

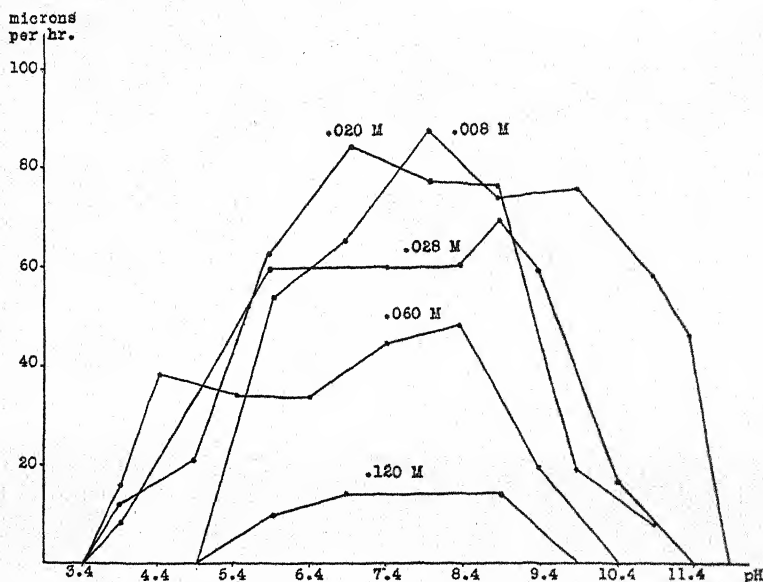


TEXT FIG. 5. Maximum root-hair elongation in calcium chlorid of various molar and hydrogen-ion concentrations.

TABLE 9. Root-hair Elongation in 0.020 M CaCl_2

pH	Average Microns per Hour	Av.	Max.
3.4	4.7; 2.2; 2.5; 1.3; 2.2	2.6	4.7
3.9	1.7; 18.0; 16.8; 37.0; 16.8; 1.7; 3.1; 3.1; 4.4; 5.0	10.8	37.0
4.9	3.4; 3.4; 3.4; 4.0; 2.2; 0.8; 1.7; 0.8; 66.2; 2.2; 33.3; 58.2; 57.9; 3.1; 42.0; 66.2	21.8	66.2
5.9	80.9; 84.9; 96.0; 59.7; 70.7; 42.6; 38.2; 50.7; 44.5; 47.6; 46.7; 66.2; 75.2; 73.1	62.6	96.0
6.9	101.7; 109.8; 72.8; 59.7; 73.1; 71.5; 108.5; 92.1; 78.7; 73.7; 107.9; 91.1; 71.8; 86.8	85.0	109.8
7.9	61.2; 56.9; 70.9; 32.7; 84.9; 50.7; 57.5; 63.8; 96.7; 95.4; 73.1; 101.4; 96.4; 81.5; 77.7; 99.6	75.8	101.4
8.9	62.8; 89.3; 88.0; 77.7; 98.6; 96.4; 82.7; 85.2; 59.1; 46.7; 70.3; 48.5; 40.5; 33.5; 65.3; 31.1	73.5	98.6
9.9	29.8; 33.0; 28.0; 22.4; 12.7; 20.5; 19.8; 14.0; 7.2	21.0	33.0
10.9	22.7; 31.1; 5.9; 4.0; 10.3; 9.0; 6.5; 2.0	11.3	31.1
11.4	1.7; 1.0; 0.8; 1.0	1.1	1.7

Also one hair in 9.9 grew for only the first half-hour, and its readings were therefore not included. The graphs obtained for the maximum (text fig. 5) and the average (text fig. 6) are of the same type as the average for 0.008 *M*. The optimum pH is, however, at 6.9 instead of 7.9; and, in fact, the whole graph is shifted toward the acid side.



TEXT FIG. 6. Average root-hair elongation in calcium chlorid of various molar and hydrogen-ion concentrations.

TABLE 10. *Root-hair Elongation in 0.028 M CaCl₂*

pH	Average Microns per Hour	Av.	Max.
3.4	16 hairs measured; no growth in any of them.	0	0
3.9	4.7; 2.2; 14.6; 18.7; 14.0; 6.2; 9.3; 1.3; 2.2; 10.9; 5.9; 2.5; 5.6	8.3	18.7
5.9	70.6; 55.0; 51.3; 33.9; 42.6; 55.0; 40.8; 64.9; 63.1; 73.1; 60.0; 72.2; 79.9; 73.1; 71.5	60.7	79.9
7.4	44.5; 65.9; 56.9; 51.0; 62.2; 48.2; 33.3; 61.0; 68.4; 77.1; 66.2; 74.0; 54.7; 61.4; 43.6; 71.5	60.6	77.1
7.9	65.3; 58.5; 48.5; 61.6; 26.1; 27.0; 62.2; 36.4; 31.1; 32.2; 30.2	43.6	65.3
8.4	47.6; 41.1; 44.5; 56.9; 39.5; 49.1; 47.3; 42.3; 57.9; 45.4; 79.6; 88.6; 79.3; 59.1	61.8	88.6
8.9	61.4; 70.6; 46.7; 58.2; 49.8; 73.7; 70.0; 88.9; 84.9; 84.9; 89.3; 81.5; 72.2	71.7	89.3
9.4	84.9; 99.2; 86.9; 80.9; 73.1; 82.1; 84.3; 31.1; 28.9; 32.0; 41.4; 33.6; 41.1; 43.2; 46.7	59.3	99.2
10.4	47.3; 49.8; 47.0; 39.5; 42.6; 44.5; 5.0; 10.0; 13.0; 9.0; 7.0; 12.0; 1.0; 4.0	25.4	49.8
11.4	14 hairs measured; no growth in any of them.	0	0

From the data given in table 10 there are omitted the readings on eleven root hairs. Two of these were in 3.9 and did not grow at all. One was in 7.9, and it stopped growing during the first hour. Five of them were in 8.4; their averages for the periods that they grew were 40 and 50 microns per hour, respectively, which was nearly average for the group. Three of the hairs excluded were in 8.9; they also grew 40 to 50 microns per hour, but stopped. The graphs for the maximum (text fig. 5) and the average (text fig. 6) are of the same form as those in 0.020, but they are reversed, the optimum being on the alkaline side and the flat portion on the acid side. The range is, however, about the same.

TABLE 11. *Root-hair Elongation in 0.060 M CaCl₂*

pH	Average Microns per Hour	Av.	Max.
3.4	6.1; 15 other hairs showed no growth.	0.4	6.1
3.9	28.9; 35.1; 14.0; 20.2; 24.3; 13.4; 19.6; 21.8; 29.9; 14.0; 18.0; 12.4; 12.4; 13.1; 13.4; 6.2; 11.8; 14.6; 11.5; 11.5; 15.6; 15.6; 15.6	16.2	35.1
4.4	38.6; 31.7; 25.6; 28.0; 38.8; 34.2; 39.9; 27.0; 46.3; 41.7; 40.5; 39.9; 42.6; 44.2; 34.2; 24.9; 45.7; 44.2	35.5	46.0
5.4	42.6; 33.6; 37.3; 34.2; 43.6; 42.0; 35.8; 23.0; 22.7; 28.0; 25.8; 24.6	32.8	43.6
6.4	30.2; 37.6; 42.6; 31.1; 21.5; 22.7; 37.6; 31.1; 25.5; 32.7; 36.7; 34.8; 27.7; 31.1; 42.0	32.5	42.6
7.4	51.3; 39.5; 36.4; 33.3; 47.3; 43.2; 51.0; 53.5; 48.2	44.9	53.5
8.4	51.6; 56.0; 50.7; 31.7; 50.4; 40.5; 49.8; 45.7; 32.7; 47.3; 51.3; 54.4; 65.9	48.3	65.9
9.4	34.2; 36.1; 20.8; 21.5; 24.9; 10.0; 10.8; 11.5; 13.1; 20.5; 10.8	19.5	36.1
10.4	10 hairs measured; no growth in any of them.	0	0

In the data presented in table 11, the readings on ten root hairs measured were not included. Two of these were in 5.4; one of them did not grow at all, and the other grew at an average rate during a little more than two hours. One hair in 6.4 behaved in this same way. Four hairs in 7.4 stopped growing during the last hour, but showed a slower growth than the average

TABLE 12. *Root-hair Elongation in 0.120 M CaCl₂*

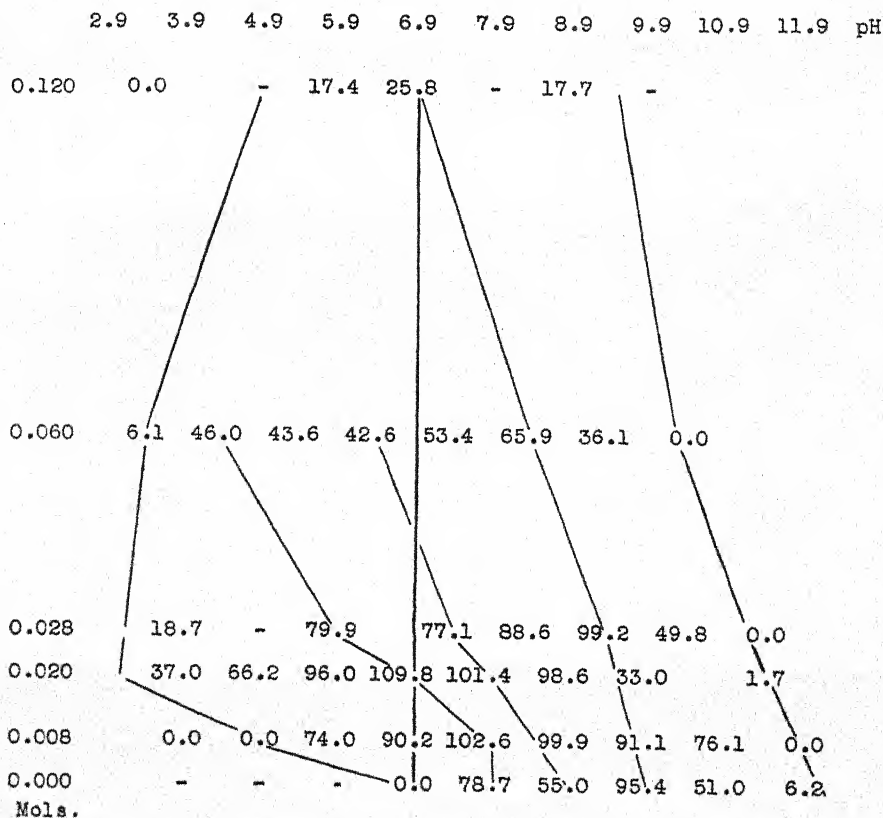
pH	Average Microns per Hour	Av.	Max.
3.4	No aquatic root hairs formed.	0	0
5.9	10.0; 4.7; 7.5; 10.0; 8.4; 6.0; 3.7; 7.8; 13.4; 8.4; 10.8; 7.5; 16.2; 16.2; 16.9; 16.9; 15.6; 15.2; 17.4; 11.8; 12.7; 10.0; 11.8; 14.3	11.0	17.4
5.9	11.5; 7.8; 10.8; 16.2; 17.1; 17.4; 9.6; 11.5; 7.8; 10.8; 10.3; 10.3; 8.1; 3.7; 7.5; 7.8; 5.9; 4.4; 5.9; 2.5	8.8	17.4
6.9	9.3; 13.4; 14.9; 3.7; 19.9; 19.3; 14.9; 22.7; 4.4; 23.3; 23.6; 16.5; 20.8; 18.7; 15.6; 13.4; 25.8; 11.5; 4.0; 4.7; 9.6; 11.8; 11.2; 11.2	14.3	25.8
8.9	15.6; 15.6; 17.7; 8.4; 15.2; 12.7; 13.7; 16.2; 6.8; 12.4; 6.5; 17.1	13.1	17.7

during the first two hours. Three hairs in 8.4 and three in 9.4 did likewise. The graphs for the maximum (text fig. 5) and the average (text fig. 6) both are definitely bimodal, with the greater optimum on the alkaline side. The general form of the curves is like that in 0.028, but lower. The acid limit is the same, but the alkaline limit is at a higher hydrogen-ion concentration.

In the data given in table 12 there are included readings on twenty-nine root hairs which stopped elongating during the last half hour of the three-hour period. Nine of these were in the first series at 5.9, and ten in the second.



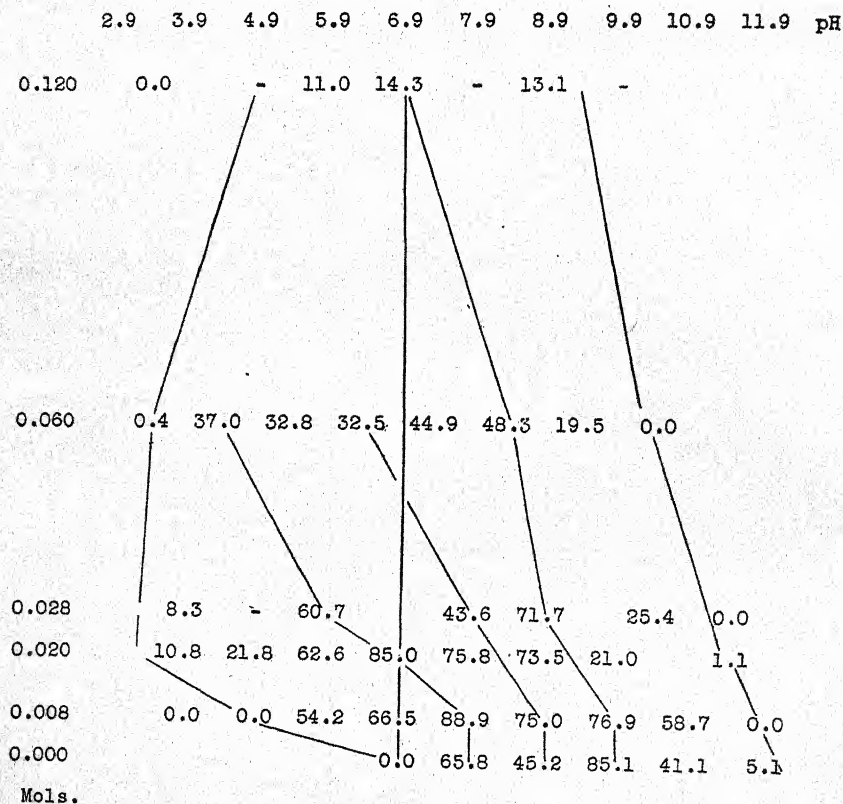
TEXT FIG. 7. Three-dimensional graph. Vertical parallel lines indicate pH units. Horizontal graphs refer to respective molar concentrations. Uprights represent rate of root-hair elongation. The photograph is made from such an angle that the alkaline optimum and the median minimum appear as straight lines. The white area represents acid solutions; the gray, alkaline; and the black, lethal concentrations. This model is based on text figures 4 and 5.



TEXT FIG. 8. The pH-molar-rate relation for maximum root-hair elongation of Georgia collards in calcium chlorid. The median straight vertical line indicates the approximate location of neutrality (pH 6.9). The boundary line on the right-hand side represents the alkaline limit of root-hair elongation; that is, at hydrogen-ion concentrations lower (pH values higher) than those falling on this line, no root-hair elongation occurs during the period of 13 to 16 hours after immersion. The boundary line on the left-hand side represents the acid limit of root-hair elongation; that is, at higher hydrogen-ion concentrations no root-hair elongation occurs within this interval. The oblique line nearest the alkaline limit is the alkaline optimum; that is, it connects a series of points mostly on the alkaline side which support a higher rate of growth than hydrogen-ion concentrations slightly higher or lower. The oblique line near the acid limit is the acid optimum. The line between the acid and alkaline optima is the median minimum. It connects the points between the acid and alkaline optima which show the slowest growth in each molar concentration studied.

In this diagram and in the following one (text fig. 9) which is constructed in a similar manner, the numbers representing growth rate are so placed that the decimal point in each case is located at the intersection of the molar concentration and the pH value lines which correspond to those used in the solution supporting that growth rate. The dashes (-) used in the diagram indicate concentrations which were not used, and hence for which no data is available. From this diagram readings for seven solutions which were studied are omitted, because of danger of confusion due to crowding of numbers. They were for 0.028 *M* at pH 3.5, 7.9, and 8.9; 0.020 *M* at pH 3.5 and 10.9; 0.008 *M* at pH 11.5; 0.000 at pH 8.5. The two omitted readings at 3.5 were about equal to that of 0.060 *M* at pH 3.5. The other five omitted readings were intermediate between those of the next lower and next higher

Three of them were in 8.9. The exclusion of these data would not alter the results appreciably. The other seven were in 6.9. They were the last hairs recorded above and grew slowly, so that their exclusion would raise the average for that pH to 17.4, which is probably more exact. Two series of 5.9 were run, each with a large number of root hairs inasmuch as they are close together in these high concentrations, in order to test the effect of temperature. In the first set the morning temperature was 20° C.; in the

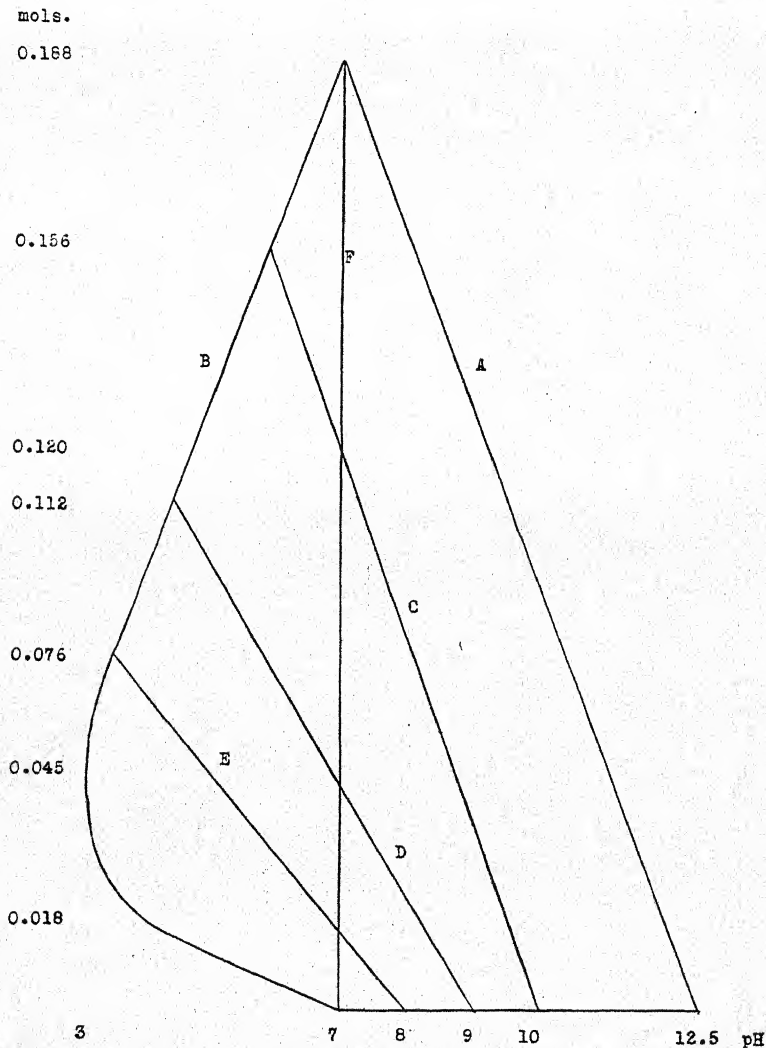


TEXT FIG. 9. Similar to text figure 8, but giving the data of the average root-hair elongation instead of the maximum, in each case. The data obtained but omitted from this diagram were for: 0.060 *M* at pH 3.9; 0.028 *M* at pH 3.5, 7.5, 8.5, and 9.5; 0.020 *M* at pH 3.5 and 10.9; 0.008 *M* at pH 11.5; 0.000 at pH 8.5. The statements made regarding the omissions in text figure 8 apply here also. 0.000 calcium chlorid refers to $\text{Ca}(\text{OH})_2$ solutions.

second set it was 18° C. The results show no effect of temperature, and a close similarity in results when the experiment was repeated. The graphs for 0.120 are monomodal with an optimum at the neutral point, and a little more tolerance of alkali than of acid.

The graphs given in text figures 5 and 6 may be better correlated and

interpreted if represented, respectively, in the form of three dimensions. This has been done for the former set of graphs (text fig. 5) by cutting out the several graphs and disposing them erect upon a plane in the order of the molar concentrations which they represent, and at intervals corresponding to these molar differences. The model is shown in text figure 7. Here the ordinates (the height of the graphs), as before, represent the rate of root-hair



TEXT FIG. 10. Idealized floor-plan of tri-dimensional graph, showing the pH and molar ranges, and indicating the location of the median minimum, the acid and alkaline optima, and neutrality. The lines on the map are lettered as follows: *A*, alkaline limit; *B*, acid limit; *C*, alkaline optimum; *D*, median minimum; *E*, acid optimum; *F*, neutrality. The molar concentrations given on this map are not those investigated, but are intended to refer to approximately the concentration at which two or more of these lines intersect.

elongation. There are, however, now two abscissae. The width of the plane (the horizontal dimension of the individual graphs) represents, as before, the hydrogen-ion concentration of the solutions. The length of the plane (the distances separating the respective graphs) represents the molar concentration.

Another method of representing the same relationship, though not so graphically, is given in text figures 8 and 9. Here the two abscissae are shown graphically, and the ordinates are indicated by the numbers. A vertical line has been introduced to represent neutrality (pH 7.0). Lines have also been drawn connecting the numbers representing, respectively, the alkaline limit, the acid limit, the alkaline optimum, the acid optimum, and the median minimum.

A third method of representation of this relationship consists in the omission of the numbers from the figures just described, the projection of the lines until they meet, and an idealizing of the figure by smoothing the lines. This has been done in text figure 10. It consists of a map indicating the tolerance of the plant for hydrogen-ion and molar concentrations of this salt.

From these data it is evident that root hairs of *Georgia collards* are able to grow in a wide range of hydrogen-ion concentrations, varying from pH 3.4 on the acid side to 11.9 on the alkaline side. However, this entire range is not attained in any one molar concentration. The extreme alkaline limit is reached only in calcium hydroxid, and the acid limit in moderate concentrations of calcium chlorid. The portion of the pH scale bounded by these limits includes practically the entire biological range. This serves as very strong evidence of the great powers of the plant to adapt itself to a wide range of acidities and alkalinities in the medium in which it finds itself. Except under exceedingly extreme conditions it would seem that the root has the ability to produce root hairs in any hydrogen-ion concentration. This ability is only potential, however, as the actual production may be inhibited by other factors, such as too much calcium chlorid in the case of an alkaline milieu, or too little or too much calcium in the case of acid media.

With increase of salt concentrations (CaCl_2) the alkaline limit is seen to shift gradually toward neutrality, reaching that point on the pH scale theoretically at 0.188 *M* (text fig. 10). Assuming that alkalinity is due to the presence of hydroxyl ions and not simply to a scarcity of hydrogen ions, we must conclude that in the solutions represented by the neutral line there are only two ions present, (Ca) and (Cl); in the solutions represented by the lower line in the chart there are two ions, (Ca) and (OH). In all other solutions represented by this chart there are three ions: on the alkaline side of the neutral line (Ca), (Cl) and (OH); on the acid side (Ca), (Cl) and (H). The highest pH value permitting growth is attained in solutions with only two ions, (Ca) and (OH). The highest molar concentration is attained in solutions with only two ions, (Ca) and (OH). Both the pH limit and the molar limit are less in solutions containing three ions. In all of these

solutions calcium is present. It would therefore seem that the shifting of the alkaline limit toward neutrality with increased molar concentration is due to the combined inhibitory effects of (Cl) and (OH) ions. That is, if hydroxyl ions are added to solutions high in chlorine ions, the solution is rendered lethal; also, if chlorine ions are added to solutions high in hydroxyl ions, the solution is rendered unfit for the production of root hairs. Inasmuch as the growth curves (3, 5, 6) for both molar and hydrogen-ion concentrations show a gradual decline to the respective limits, it may be concluded that high concentrations of either (Cl) ions or (OH) ions tend to be injurious to growth. Furthermore there is no antagonism of these two anions for each other, so that in extreme concentrations there is an additive injurious effect.

On the acid side a different situation obtains. The acid limit shifts away from neutrality with rise in concentration of calcium chlorid up to 0.020 molar solution. Above this it remains quite constantly near 3.4, up to 0.060 *M* and then appears to diverge toward neutrality again. Assuming that at 0.120 *M* the acid limit is at 4.9 (text figs. 8 and 9), as seems reasonable from the data obtained, then the acid limit will theoretically reach neutrality at 0.188 *M*. In other words, growth of root hairs does not occur in solutions high in calcium ions and high in hydrogen ions, nor in solutions very low in calcium ions and high in hydrogen ions. It is only in moderate salt concentrations that the extremely acid solutions are not lethal. The calcium ion evidently antagonizes the injurious effect of high hydrogen-ion concentration, because in the absence or scarcity of calcium very acid solutions are lethal. The reverse does not, however, seem to be true. Solutions which are high in both calcium and hydrogen ions are also lethal. It would seem therefore that hydrogen ions do not antagonize the injurious effect of high calcium concentrations. On the other hand the injurious effect here seems to be entirely additive, just as in the case of (Cl) and (OH) ions on the alkaline side. It is possible that the failure of the (H) ion to antagonize the (Ca) ion in high concentrations of the latter may result from the presence of a high concentration of the (Cl) ion as well.

It is observed that the form of the chart (text fig. 10) representing the pH and molar ranges is that of an isosceles triangle for concentrations above 0.060. The altitude of this triangle is the neutral line. In other words, at high concentrations the pH range is equal on the acid and alkaline sides. At low concentrations this is not true. However, if the base line for calcium hydroxid be projected until it meets the projection of the acid limit of high concentrations, the resultant figure will also be an isosceles triangle. In other words, the plant is equally tolerant of acids and alkalies, except in dilute acid solutions. This latter we have explained as being due to the absence of calcium to antagonize the injurious effect of the hydrogen ion.

Although we can say that with this exception this plant is equally tolerant of acids and alkalies, we do not mean that the rate of growth of root

hairs is equal in a given molar concentration at equal departures from the neutral point on the acid and alkaline sides, respectively. The statement is meant to refer to the viable pH and molar ranges of growth only, as contrasted with lethal concentrations. The curves (5, 6) showing variations in growth rate in different hydrogen-ion concentrations at given molar concentrations are far from symmetrical on either side of neutrality. They are all platycurtic with a tendency to very abrupt decline near the acid and alkaline limits, respectively. There is also a tendency to be bimodal in the median concentrations (0.020, 0.028, 0.060), though the curve for very high concentrations (0.120) is monomodal. When the graphs for the respective molar concentrations are spaced as in the model shown in text figure 7, it becomes apparent that the monomodal form of the high concentration is the result of the theoretical median minimum for this molar concentration, occurring at a pH value which is lethal to root hairs. It is to be noted that in the data based upon average growth rate (6) the curve for 0.008 also is bimodal; but that based upon the maximum readings for this concentration (5) is monomodal. The absence of two peaks here may be due to the close proximity of the two optima. It is, however, likely that further study at shorter pH intervals in this concentration would reveal a median minimum at about 8.4, and an acid optimum at about 7.4. This is indicated by a comparison of text figure 8 with the idealized presentation in text figure 10, in which the lines for the acid optimum and for the median minimum have been straightened in this region.

A consideration of the model reveals that there is a definite relationship between the modes of the respective molar graphs. In fact, it becomes apparent that in addition to the neutral point there are three critical points on the pH scale. These may be designated as the alkaline optimum, the median minimum, and the acid optimum. These three points are not the same for different molar concentrations of the salt, but they all shift toward the acid side with increased concentration. The alkaline optimum shifts to the same degree as the alkaline limit, reaching neutrality at 0.120, and theoretically intersecting the acid limit at 0.156 *M*. The acid optimum shifts, however, more abruptly, intersecting the neutral line at 0.018 and the acid limit at 0.076. The median minimum takes an intermediate position at all concentrations intersecting the neutral line at 0.045 and the acid limit at 0.112 *M*. In other words, the lines for the acid optimum, the alkaline optimum, and the median minimum might be projected below the base line to meet at a focal point, represented by -0.045 *M* at pH 11.

The direction of the alkaline optimum may be attributed to the same cause as that of the alkaline limit, namely to the additive injurious effect of chlorine and hydroxyl ions. The departure of the acid optimum from a position parallel to this line indicates that it is controlled to some extent by the antagonism of calcium for hydrogen ions; hence it tends to be parallel to the acid limit at low molar concentrations. As far as calcium chlorid is

concerned the acid optimum is higher than the alkaline optimum at the lower concentrations, that is, at concentrations in which it is not on the acid side of neutrality. In the calcium hydroxid curve, however, the alkaline optimum is the greater. The acid optimum may, however, be lower in calcium hydroxid because it is only one pH unit removed from a lethal concentration, namely 6.9; whereas the alkaline optimum is at least two pH units removed from a lethal concentration, 11.9.

The graphs also show that in all molar concentrations below 0.120 the root hairs of collards may grow more rapidly (during the period of 13 to 16 hours after immersion) in neutral or alkaline solutions than in any acid solution at the same concentration. The most growth of all occurs in a neutral or slightly alkaline solution. However, in some slightly acid solutions they grow better than in an alkaline solution just on the other side of neutrality, as, for instance, in 0.028 *M* at pH 5.9 and 7.9, respectively.

It is apparent from these data and graphs that there is a direct relationship between hydrogen-ion concentration and salt concentration. The optimum concentration of the salt depends upon the hydrogen-ion concentration of the solution. At a pH of 7.9, for instance, growth of root hairs is more rapid in a molar solution of 0.008 than in 0.020, but at pH 6.9 the reverse is true. It is also true that the optimum pH depends upon the molar concentration of the salt. In 0.008 CaCl_2 growth is best at pH 7.9, in 0.020 *M* at 6.9, in 0.028 at 9.4, and in 0.060 at 8.4.

In connection with the evaluation of these results it becomes desirable to know how much, if any, change occurred in the hydrogen-ion concentration of the solution while it was flowing from the flask through the chamber in which the radicles were immersed and thence into the overflow tube. Such determinations were made for all hydrogen-ion concentrations studied in 0.008 and 0.020 molar solutions, and for 0.028 *M* at pH 3.4 and 3.9. For these last two solutions the solution passing out of the overflow tube had the same hydrogen-ion concentration, according to the indicator method used, as the solution in the flask. The data for 0.008 and 0.020 solutions are presented in table 13.

TABLE 13. *Change of H-ion Concentration of Solutions*

<i>a</i> Solution: 0.008 <i>M</i> CaCl_2									
Entering.....	3.9	4.9	5.9	6.9	7.9	8.9	9.9	10.9	11.9
Leaving.....	3.9	4.9	5.9	6.4	7.4	8.4	8.4	9.4	10.9
<i>b</i> Solution: 0.020 <i>M</i> CaCl_2									
Entering.....	3.4	3.9	4.9	5.9	6.9	7.9	8.9	9.9	10.9
Leaving.....	3.4	4.4	5.4	5.4	6.4	7.4	8.4	9.4	9.4

In this table the hydrogen-ion concentration of the solution as it left the chamber in which the roots were immersed is indicated directly below the number representing the hydrogen-ion concentration of the solution as it entered the chamber.

From the data in table 13 it is apparent that solutions of a pH value of

9.9 and 10.9 are very unstable. It has also been noted that the indicators used for determining these hydrogen-ion concentrations were not very precise. It therefore appears that a less degree of dependence should be placed upon the data given over this range. It is furthermore to be noted that this doubtful range applies in our work only to the lower molar concentrations, and falls in all cases between the alkaline optimum and the alkaline limit or beyond it.

Table 13 shows that in solutions in which root hairs are growing there is a tendency for the solutions of a pH higher than 5.4 to shift downward, that is, to become more acid; and for the solutions of a pH value lower than 5.4 to become more alkaline. The extent of this change, except in the doubtful range noted above, is about one-half of one unit. The drop in pH might be attributed to the liberation of carbon dioxide by the root, making the solution more acid. The rise in pH in other solutions may be due to an exchange for calcium of some ion which makes a stronger base, as K or Na. The absence of a change in pH of the solution in cases of very little or no growth demonstrates the feasibility of using carbon-dioxide-free single-salt solutions without a buffer for the study of specific reactions of single ions.

DISCUSSION

The marked ability of the root to adjust itself to solutions of widely different hydrogen-ion concentration is an impressive feature of the above data. Root hairs of Georgia collards are here shown to be produced and to grow during the interval of 13 to 16 hours after immersion in solutions having a pH of from 3.4 to 11.9, inclusive. Bryan (139) reports that the pH range for alfalfa and clover in balanced solutions is 4 to 10, and for wheat and oats 3 to 10. This is almost precisely the range which we have found in 0.060 calcium chlorid. It is doubtful, in fact, if any organism or organ, other than the root, can adapt itself to such a wide diversity of alkalinity and acidity. Morgulis, Beber, and Rabkin (164) found the range for catalase activity to be pH 2 to 14; but this can hardly be regarded as a vital phenomenon. Some organisms can withstand a higher hydrogen-ion concentration, but they do not function so far on the alkaline side. Mrs. Brooks (137) found that the hydrogen-ion range for carbon dioxide production of *Bacillus subtilis* extends from 8.4 to about 1.3, and that for *B. tuberculosis* (138) reaches a pH of 1.0. Gustafson (148) found that *Penicillium* carries on respiration below pH 3.4. He says that a solution of 2.65 causes a temporary rise in rate; one between 1.95 and 1.10 results in a final drop which is irreversible. The pH range for root hairs of collards seems all the more remarkable in that the process studied is growth, which is usually conceived of as the resultant of a complicated series of metabolic changes, the interruption of any one of which might cause its cessation. Itano and Neill (153) report that *Bacillus subtilis* germinates between pH 5 and 10, but not beyond these limits. Miss Addoms (133) found that at a pH of 4.0

or below the protoplasm of root hairs of wheat coagulated. Mrs. Lewis (154) likewise reported that the cytoplasm and nuclei of embryonic cells coagulates at pH 4.6. The root hairs of Georgia collards which had formed in air were found to coagulate in many instances in solutions of a pH of 3.9 or below. Nevertheless other root hairs were produced in a number of solutions of this degree of acidity. Bakke and Erdman (136) found the acid limit for the growth of wheat in balanced solutions to be pH 4.3. As indicated in our work above, the acid limit varies with salt concentration. It is therefore not now possible to make an accurate comparison of *Triticum* and *Brassica* as to their pH-molar range. It seems almost certain that a study of other genera, and perhaps species, will yield charts of different form and location on the pH scale from that given above (text fig. 10). Hoagland (149), for instance, reports that barley is more injured in alkaline than in acid solutions of the same divergence from neutrality. It was shown above that for collards in moderate salt concentrations the effects of acids and alkalies are in general equivalent; but that in low concentrations the acids are the more injurious.

Studies of this type have a direct application to the soil problem in its relation to the geographical distribution of plants and to the cultivation of economic crops. Arrhenius (135) states that soils vary in hydrogen-ion concentration from pH 2 to pH 12. Gillespie (147) found that the pH of 18 soils in Maine, Maryland, and Virginia fell between 4.4 and 7.0, inclusive, and that four soils in Utah and Montana varied from 8.0 to 8.6. He found that a soil of pH 4.5 was not injurious to potatoes. Hoagland (150) found that California peat soils are acid, varying from 4.5 to 6.0; and Olsen (165) found that those in Denmark range from 3.4 to 8.0. It thus appears that ordinarily the pH range of the soil does not exceed the ability of collards to adjust themselves to its hydrogen-ion concentration.

The platycurtic form of the graphs plotted along the pH scale presented in this paper are in harmony with those secured by others using different material and methods. Cohen and Clark (143) report that bacteria grow well in a wide range of hydrogen-ion concentrations varying from pH 5 to 8, but that the curve drops off suddenly on both sides beyond this range. Most of their graphs for short periods, however, are either leptocurtic or mesocurtic. Morgulis, Beber, and Rabkin (164) secured platycurtic curves entirely in their work on catalase activity. Hopkins and Wann (11), in their recent work on *Chlorella*, have secured some curves which are almost identical in form with those given in this paper, though they are not continued to the alkaline limit. Hoagland and Davis (152) report that *Nitella* is not injured by pH concentrations between 5.0 and 9.4, inclusive, but that marked injury is shown below 4.4. Reed and Haas (85) found that a pH of 8 or 9 is not harmful to walnut seedlings, if calcium is present, but more than 9 is toxic.

Not only is the platycurtic curve the one usually obtained for the effects

of varying hydrogen-ion concentrations upon biological phenomena, but such curves are perhaps even more characteristically bimodal as well. In the discussion in the third paper of this series in which such a graph was secured for the effect of calcium hydroxid, reference was made to Robbins's papers summarizing the literature furnishing such bimodal pH curves. The results which are now presented upon different concentrations of calcium chlorid are in harmony with these findings, though it is also shown that under certain conditions, especially in high concentrations, a monomodal pH curve may exist, which is definitely correlated with the bimodal curves at other concentrations.

In this connection special attention should be paid to the work of Arrhenius (135), who obtained bimodal curves for different hydrogen-ion concentrations in water, sand, and soil cultures, respectively. He found that wheat and radish germinate in pH 3 to 10, but that the former does not continue to grow below 4, nor the latter below 5. He reports that plants at the time of germination and in the early stages of development are more tolerant of variations in hydrogen-ion concentration than at maturity. He interprets his results as showing that the intake of calcium is slightly depressed on the alkaline side, and that calcium, nitrate, and sulphate ions are absorbed most in hydrogen-ion concentrations at which there is minimum growth; while potassium, magnesium, and phosphate ions are absorbed in solutions supporting a maximum growth. These results are not in harmony with our findings or interpretation. It is to be noted, however, that Arrhenius was measuring growth not by the rate of root-hair elongation, but by grosser methods, such as dry weight increase and height of plants.

It is quite likely that much of the difficulty which has been encountered in the study of the effects of hydrogen-ion concentration upon growth in balanced solutions has been due to the ionic complexity of the solution, on the one hand, and to the physiological complexity of the criteria, on the other. Duggar (146) made an extensive study of standard and other balanced solutions with a view to ascertaining the relation of hydrogen-ion concentration to their efficacy. He decided that there is probably no best solution; but that satisfactory results are secured over a wide range of ionic and salt proportions. He obtained good growth in solutions having hydrogen-ion concentrations from pH 3.4 to 8.6, and suggests that the effect of varying hydrogen-ion concentration may be attributable in part to its effect upon the solubility of phosphates. Arrhenius (135) also refers to the effect of hydrogen-ion concentration upon the solubility of the salts. However, by reducing the problem to a cellular basis and by using only a single soluble salt, it has been shown above that mutual ionic precipitation is not to be considered a prominent factor in explaining these phenomena.

Arrhenius (135) also calls attention to the possibility that there may be a change in permeability with change in hydrogen-ion concentration. Michealis (162) has just published a paper in which he shows that this is

most certainly the case. Osterhout (166, 167) found that the permeability of *Laminaria*, as measured by electrical conductivity, increases in alkaline media and decreases in acids. Arrhenius (134) earlier reported experiments with radish and wheat in water cultures, in which he found that the former gives two optima for the absorption of water, phosphates, nitrates, sulphates, calcium, and potassium: one on the alkaline side at pH 8, and the other on the acid, at pH 5 or 6. Between these two there is a median minimum which, for the absorption of water, phosphates, and sulphates, is at pH 7, and for calcium and potassium at pH 6. Pearsall (168) found that growth is slightly more rapid on the alkaline side of the median minimum, which is in harmony with the findings presented above, except for low concentrations of calcium chlorid.

Robbins (126) has been the most active in interpreting this median minimum as the isoelectric point of the constituent proteins. His interpretation is supported by the similarity of the bimodal curve for physiological effects of hydrogen-ion concentrations to the bimodal curve secured by Loeb (117) and others for certain physical phenomena, such as the swelling of gelatin. However, it is a matter of some question as to how far biologists are justified in depending upon the similarity of physiological to physico-chemical graphs as a basis for the interpretation of results. Physico-chemical phenomena are so numerous, physiological phenomena so complex, and distinct types of mathematical graphs so few, that any such comparison should at least be definitely recognized as only a working hypothesis. Pearsall (168) leans toward an interpretation similar to that of Robbins. He concludes that within certain limits the farther the hydrogen-ion concentration is from the isoelectric point, the more rapid will be absorption and hence cell enlargement and growth. It is to be recognized that growth as he measured it is far more than cell enlargement, and that in his interpretation he adopts the view of Walter (102) that cell enlargement is the consequence of increase in cell turgor, rather than the view of Ursprung and Blum (101) that it is the result of deposition of wall material, cell turgor being at a minimum rather than at a maximum in the enlarging cell.

Robbins (172) has more recently sought to substantiate his interpretation by the use of methods other than the staining previously employed. By ascertaining the change in weight in buffer mixtures, he secures a critical point of 6.4 instead of 6.0. From a study of the ash content, he reports no definite results. Some of his results, he states, indicate that instead of there being one protein with its isoelectric point at about 6.0, there may be two, one having an isoelectric point of 4.5 and the other of about 8.0.

It is precisely in this connection that Robbins's interpretation seems to encounter one of its greatest difficulties. While the isoelectric point of a given protein can be determined with some degree of accuracy, the isoelectric points of a mixture of many proteins is quite certain to be elusive. Robertson (171) has determined the isoelectric points of a number of amino acids, and

finds that they range from 2.76 to 10.97. Pearsall (168) believes that most vegetable proteins have an isoelectric point of about 4.5, and recognizes that plant tissues show other minimum points of swelling than the isoelectric point. Pearsall and Ewing (169) determined the isoelectric points of a considerable number of plant proteins and found that they range from 3.2 to 5.5.

It seems exceedingly difficult, if not impossible, to harmonize the results given above for growth of root hairs in calcium chlorid with the concept of the median minimum as the isoelectric point of the constituent proteins. With increase in the molar concentration of the solution the median minimum shifts from pH 8.9 to pH 4.4. In like manner Lovgren (158) found that the optimum hydrogen-ion concentration for the activity of urease shifts with increase in concentration of the substrate. It could hardly be imagined that the isoelectric point of the constituent proteins would change so much with change in molar concentration of the medium. In fact, the isoelectric point of the proteins must be quite independent of the molar concentration of the solution. It is quite likely, however, that in its shift along the pH scale the median minimum will happen in some molar concentration of the salt to be the isoelectric point of the protein. This would be the case in collards if the isoelectric point of their proteins is between 8.9 and 4.4. Consequently even the demonstration that the median minimum in a given solution is at the same pH as the isoelectric point, independently determined, does not constitute proof, or even evidence, that the median minimum is a satisfactory criterion of the isoelectric point.

Truog and Meacham (175) have reported that the expressed sap of *Brassica Napus* has a pH of 5.10 on an unlimed acid soil and of 5.18 on a limed acid-soil. Of the 42 plants for which they measured the hydrogen-ion concentration of the expressed sap, there was a range of variation from pH 2.2 in citrus fruits to 6.5 in *Mimosa*. Chibnall (141) has more recently studied the pH of the expressed sap of leaves, and finds that they vary from 3.02 in grape to 6.57 in spinach. He does not believe that the hydrogen-ion concentration of the cell sap is identical with that of the isoelectric point of the constituent proteins; in fact, he maintains that such a situation could not exist, as there would result a precipitation which would cause death.

Of interest in this connection are the findings of Arrhenius (135) and Hoagland (150) as to the stability of the pH of the cell sap with change in hydrogen-ion concentration of the medium. The former found that with a change in the pH of the medium from 3 to 10, there was an alteration of the pH of the cell sap in the leaf epidermis of wheat of not over 0.1 pH, in the guard cells of not over 0.3 pH, and in the root cells of not over 0.5 pH. Hoagland found that the tops of barley varied 0.3 pH and the roots 0.9 pH with a change in the milieu from 4.9 to 6.8. Crozier (144) found that the cell sap of *Valonia* may be caused to change from a pH of 5.0 to one of 6.7.

Michealis and Mostynski (163) first defined the isoelectric point as the pH

where the relation of the concentration of the hydrogen ions to the hydroxyl ions is the same as the relation of the acid dissociation constant of the protein to the basic dissociation constant; that is, where the protein anions equal the protein cations. Michealis (161) later stated that the isoelectric point is that at which the undissociated fraction of the ampholyte is at a maximum. Loeb (155, 156) stated that at the isoelectric point there are the fewest simple ions combined with the protein, below it the protein combining with anions, and above it (that is, at a higher pH) with cations. Later (157) he referred to it as the point of greatest instability of proteins, and therefore the point of the minimum viscosity, osmotic pressure, and potential difference.

According to Michealis (161) the ampholyte should raise the hydrogen-ion concentration of the milieu from a lower pH upward toward the isoelectric point, and from a higher pH downward toward the isoelectric point. Upon this basis, then, the point toward which the pH of the solution is shifted by the organism or ampholyte might be regarded as the isoelectric point. There is thus a criterion for this point, other than the median minimum of growth or metabolism. It has been shown above that the point toward which the roots of *Georgia collards* shift the hydrogen-ion concentration of the solution flowing past them is pH 5.4.

The alteration in the hydrogen-ion concentration of a solution in the presence of an organism has been reported by several investigators. Hoagland (149, 150) stated that roots of plants in solution cultures and sand cultures shift the hydrogen-ion concentration of the milieu rapidly toward neutrality. Bakke and Erdman (136) reported that the pH of their culture solutions changed during the growth of wheat from 3.75 to 5.95 in water cultures and to 6.66 in sand cultures. Robbins and Scott (128) determined the effect of different tissues upon the hydrogen-ion concentration of the solution. They found that potato at 6.5 or higher shifts the pH of the solution down, and at 6.14 or lower shifts it up. From this they conclude that the isoelectric point of the potato tuber is 6.4. In the same way they determined the isoelectric point of soy bean to be from 6.2 to 6.44, of *Gibberella* 6.2, of *Fusarium lycopersici* 5.4, and of *Fusarium oxysporum* 4.9. Hopkins and Wann (11) also report that the hydrogen-ion concentration of their nutrient solutions was usually lowered, if acid, or raised, if alkaline, by the growth of *Chlorella*. Hoagland (109) has recently expressed the conviction that in acid solutions more anions than cations are absorbed, and in alkaline solutions more cations than anions. Youden and Denny (177) have very recently published a thorough study of the situation in the potato tuber. They find that most of the effect upon the solution is due not to the selective absorption of ions, but to the leaching out of ions from the tissue. They find that these ions are neither protein nor colloidal, and hence conclude that such substances do not play an important part in this change of pH. They find, in harmony with Robbins and Scott, that the equilibrium

point for potato is 6.4, whereas the isoelectric point of the principal protein, tuberin, has been reported to be 4.0. It is therefore concluded that this equilibrium point is not a sound criterion of the isoelectric point of the constituent proteins.

The beneficial effects of adding lime to acid soils has attracted attention to the relation of calcium content to hydrogen-ion concentration of the soil. The addition of CaO obviously increases the calcium concentration and lowers the hydrogen-ion concentration by the formation of Ca(OH)_2 . By the model (text fig. 7) it is shown that in an acid soil free from calcium there is no growth, but the addition of a very small amount (0.008 to 0.020 *M*) of calcium chlorid changes the concentration to one which supports the optimum growth rate. The further addition of calcium, either as oxid or chlorid, serves to support a very rapid growth rate unless the amount be excessive, such as is represented by 0.060 *M* CaCl_2 or a pH of 10.

In harmony with the interpretation given above that the absence of growth in dilute acid solutions is due to the absence of calcium to antagonize the hydrogen ions, Hoagland (109) states that an acid soil may be unfavorable for plant growth simply because of lack of calcium. Arrhenius (135) also has come to attribute the beneficial effects of lime to its antagonism of acidity and not to the direct chemical effect of calcium. He contends that plants should not be classified as calciphobous and calciphilous, but that they should be grouped into acidophile and basophile categories. Cameron (47) also favored abandoning the older classification; and Truog (174) regards the more recent one as likewise misleading.

A considerable amount of evidence, in addition to the work of Arrhenius cited above, has now accumulated showing that calcium antagonizes the injurious effects of hydrogen ions. Dachnowski (145) found that the addition of salts to nutrient solutions reduces the toxic effect of acids. Prianischnikow (170) has more recently shown that calcium increases the resistance of plants to acids, finding it more antagonistic to the hydrogen ion than Mg, Mn, Ba, or Sr, and much more than K or Na. Lundegardh (159) also interpreted his results on *Gibberella* as showing that calcium protects against injury by acids. Mevius (160) has just published an extensive study of the growth of roots of pine, corn, and legumes, in which he arrives at the same conclusion. The data which we have secured upon root-hair elongation confirms this relation for low concentrations of the salt; but it is apparent that addition of calcium above a certain concentration (0.060 *M* CaCl_2) leads to the reverse effect, that is, the suspension of root-hair elongation in very acid solutions.

Mevius (160) has sought to explain this antagonism on the basis of permeability. He cites Stiles, Höber, and Kaho to the effect that calcium ions reduce permeability. On the basis of his own experiments he concludes that the hydrogen ion increases permeability, although Osterhout found that both calcium chlorid (166) and acids (167) decrease permeability. On

the basis of the root-hair work one is brought to the conclusion that the calcium ion antagonizes the hydrogen ion, but that the hydrogen ion does not antagonize the calcium ion. It would seem, therefore, that no simple explanation of an effect upon permeability is adequate. The calcium ion evidently has many effects, such as deposition in cell walls and precipitating oxalate, other than modifying permeability, and the same is doubtless even more true of the hydrogen ion.

Chambers and Reznikoff (49) find that amoebae die within 24 hours in HCl between pH 5 and 6. This is not antagonized by calcium or other chlorids. Pantin (78) finds that the more acid a solution, the more calcium is required to cause inhibition of amoeboid movement. We have found that the more acid a solution the more calcium is required to bring about cell enlargement at low concentrations.

Reed and Haas (85) conclude that calcium is antagonistic also to hydroxyl ions. They found that walnut seedlings perish quickly in $\text{Ca}(\text{OH})_2$ of pH 9 or higher, but that they continue to live for at least a week if the solution is repeatedly renewed. They interpret this injury of high pH as due to calcium starvation. We find that the hydroxyl ion is not so injurious in very low calcium chlorid as in higher concentrations. Theron (173) and Hoagland and Davis (110) found that nitrate and chlorine ions, respectively, are absorbed more rapidly by higher plants in slightly acid than in slightly alkaline solutions.

Hoagland and Davis (110) in their recent excellent discussion of the problems of absorption conclude that neither mass action, Donan's equilibrium, nor protein isoelectric points, taken singly or together, can adequately explain the distribution of ions on the inside and the outside of the cell. From this work on root hairs, it would seem that a very important consideration in this connection is the relation of the molar concentration of the salt (especially Ca) to the hydrogen-ion concentration of the milieu, as indicated by the median minimum, and also perhaps by the location of the acid and alkaline optima, respectively, on the pH scale.

It is apparent that too sweeping conclusions should not be made from so small a beginning. In this paper there has been reported the effect of only one salt upon one variety of one species of plant. In this study about 13,000 increment readings were taken upon about 700 root hairs. Similar studies, using other salts on the same plant and the same salt with other plants, should yield significant results. By the comparison of charts and graphs of the type here presented, it should be possible to make some definite contribution to our knowledge of soil problems, growth processes, absorption, and inter-ionic relations

SUMMARY IV

30. The method of preparing solutions of various hydrogen-ion concentrations of calcium chlorid and of testing them is described and discussed in detail.

31. Neither the presence of calcium oxid in the dry salt, nor the increase in molar concentration due to the addition of calcium hydroxid or hydrochloric acid to adjust the solution to the desired hydrogen-ion concentration, constituted sources of error of any consequence in this study.

32. Tables of data are given based upon over 13,000 readings on more than 700 root hairs in different concentrations of hydrogen ions and calcium chlorid. Graphs are plotted for 0.008, 0.020, 0.028, 0.060, and 0.120 molar calcium chlorid solutions, using the maximal and average readings, respectively.

33. The maximum rate of elongation of root hairs attained in any of these solutions was 109.8 microns per hour in 0.020 CaCl_2 at pH 6.9.

34. The curves plotted along the pH scale are all platycurtic, and nearly all bimodal.

35. From these curves, together with those for calcium hydroxid given in paper III of this series, there is constructed a three-dimensional graph showing the pH-molar-rate relation. From this a chart is drawn showing an idealized floor-plan of the graph, and defining the acid and alkaline limits and optima, and the medium minimum, for collards in calcium chlorid.

36. The alkaline limit shifts gradually toward neutrality with increased salt concentration.

37. The acid limit shifts with increasing salt concentration from neutrality to pH 3.4 at 0.020 M , remains there up to a concentration of 0.060 M , and then shifts back toward neutrality.

38. The alkaline optimum shifts toward the acid side in a line parallel to the alkaline limit beginning at pH 10, and crossing the neutral line at 0.120 M .

39. The median minimum, beginning at pH 9, shifts more rapidly toward the acid, crossing neutrality at 0.045 M .

40. The acid optimum, beginning at pH 8, shifts still more rapidly toward the acid, crossing neutrality at 0.018 M CaCl_2 .

41. The hydrogen-ion concentration range for root-hair elongation of Georgia collards is found to be from 3.4 to 11.9, though this range is not attained at any one molar concentration. The molar solution of calcium chlorid having the widest range is 0.020 M , namely, 3.4 to 11.4.

42. Root hairs of collards are found to grow in equally extreme acid and alkaline solutions at high concentrations (above 0.060 M); but at low concentrations they are more tolerant of alkaline than acid solutions.

43. At any given molar concentration the most rapid growth occurs in a neutral or alkaline solution, though in some slightly acid solutions growth may be more rapid than in a slightly alkaline solution of the same molar value.

44. In producing root hairs which will grow, roots exhibit the power of adaptation to a wider range of hydrogen-ion concentrations than any other

organ or organism or physiological process which has thus far been studied, though enzymes have been found to tolerate a still wider range. This range is sufficient to include almost all natural soils of the earth.

45. The platycurtic bimodal pH curve is characteristic of other organisms and processes, as shown by the work of others. It is not to be explained on the basis of mutual ionic precipitation, inasmuch as a single-salt solution was here employed; but it may be largely an effect of the ions upon permeability.

46. The median minimum is not regarded as a good criterion of the isoelectric point of the constituent proteins, inasmuch as it is found to shift so markedly toward the acid side with increased molar concentration of the solution.

47. The solution flowing past the root hairs is altered in its hydrogen-ion concentration about one-half of one pH unit in the direction of 5.4. This is interpreted as resulting from a difference in the ionic exchange between the root hairs and the milieu at high and at low hydrogen-ion concentrations. The fact that pH 5.4 is the critical hydrogen-ion concentration in this relation may be interpreted as being due to the possibility that it is the average of the isoelectric points of the constituent proteins, inasmuch as it has been shown that non-living ampholytes alter solutions in this way.

48. The growth of root hairs in very acid solutions of calcium chlorid of moderate molar concentrations, and its absence in dilute or concentrated solutions, is interpreted as due to the antagonism of calcium for the injurious effects of high hydrogen-ion concentration, and the absence of an antagonism of hydrogen ions for the injurious effects of high calcium content.

49. The growth of root hairs in alkaline solutions is interpreted as resulting from the absence of an antagonism between chlorine and hydroxyl ions.

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STUDIES IN THE CHYTRIDIALES I. THE LIFE HISTORY AND
OCCURRENCE OF *ENTOPHLYCTIS HELIOMORPHA*
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Entophlyctis heliomorpha is a unicellular chytrid of wide occurrence in dead nodal, internodal, and corticating cells of the Characeae, and like most species of this genus so far described it is intracellular. The germ tube of the zoöspore penetrates the wall of the host cell and develops into a globular sporangium. Within the sporangia are developed from two to fifty zoöspores which when mature escape to the outside of the host through a long sporangial tube or neck. After swimming about from thirty minutes to two hours they generally come to rest on the wall of the host cell and proceed to reinfect the host. This may continue for a considerable length of time, until a host cell is filled with sporangia. Several thousand sporangia may be found in a single long internodal cell. Resting spores are subsequently developed in the host cell in the same manner as are sporangia.

The first account of this chytrid was given by Dangeard in two brief descriptions in 1886 in which he placed it in the genus *Chytridium* under the name *C. helioformis*. In these two accounts he gives no figures but describes the sporangia as spherical, 10 to 20 microns in diameter, provided with six or seven rhizoid trunks; zoöspores uniciliate, spherical, 3 microns in diameter and escaping to the outside of the host through a sporangial neck; resting spores spherical with several rhizoid trunks. Dangeard described it briefly again in 1888 as occurring in cells of *Nitella*, *Chara*, and *Vaucheria*, called it *E. heliomorphum*, and gave four figures of sporangia, escaping zoöspores, and a resting spore. In 1892 Fischer combined the Rhizidiaceae and Polyphagaceae into the new family Sporochytriaceae and segregated all species of A. Braun's genus *Rhizidium* whose sporangia, rhizoids, and resting spores develop within the host cell in the new genus *Entophlyctis*. He included the chytrid found by Dangeard in this genus and changed its name to *E. heliomorpha*. In his classification of the Chytridiales Schroeter (1897) reestablishes the family Rhizidiaceae and retains the genus *Entophlyctis*, but limits it to the intracellular or endophytic species whose sporangia have no apophysis (Wurzelblase). Neither Fischer nor Schroeter gives any figures showing the life history of *E. heliomorpha*.

Since the time of Dangeard's observations little attention has been given to the occurrence of this intracellular chytrid. I have been unable so far to find any reference in the literature to its occurrence in America, and since the stages in its life history were only partly figured by Dangeard a more

complete description of its life cycle seems well worth while. A more detailed study of nuclear division, cleavage in the sporangium, penetration of the germ tube into the host cell, its pathogenicity, and other problems connected with its life history is now being made.

STRUCTURE AND BEHAVIOR OF THE ZOÖSPORES

The zoöspores of *E. heliomorpha* are generally spherical in shape, uninucleate, 3 to 4 microns in diameter, with a single posteriorly attached flagellum. As is shown in Plate I, figure 1, the flagellum may be fully eight times as long as the diameter of the spore. The most conspicuous structure in the living zoöspore is a large, round, highly refractive droplet or body generally lying in the center of the spore or slightly displaced toward the region where the flagellum is attached. Such bodies are generally present in the zoöspores of the Rhizidiaceae and were frequently mistaken by the early observers for the nucleus. The nucleus is generally invisible because of its optical homogeneity, but may sometimes be seen in the center of the spore or to one side of the refractive body as is shown in figure 1. These zoöspores appear to be identical in structure and appearance with those of *E. Confervae glomeratae* and *E. Cienkowskiana* described by Cienkowski (1857, fig. 1) and Zopf (1884).

After escaping from the sporangium the zoöspores dart back and forth with great rapidity. In water mounts they may easily be distinguished from other motile organisms of the same size and appearance by this habit of movement. They may dart forward with great rapidity, come to an abrupt stop, and then dart off again. The flagellum is always carried posteriorly, as was described and figured by Dangeard, and the zoöspores are driven forward by its continual beating from side to side. If the zoöspores are obstructed or get caught or trapped in a position in which it is impossible for them to swim about they may become amoeboid in shape and movement. They may thrust out pseudopodia in various directions as if "feeling" for some means of escape. Figures 20 to 23 show the changes in shape of a zoöspore in trying to free itself from a tight position between the cover glass and slide. As soon as this spore was freed it immediately rounded up and darted off.

GERMINATION OF THE ZOÖSPORES

After swimming about from thirty minutes to two hours the zoöspores generally lose their flagella, come to rest, and germinate. Shortly after coming to rest, and before germinating they may again become sluggishly active with amoeboid movements. In a few instances I have observed zoöspores germinating while the flagellum was still attached, but such cases appear to be rare. The zoöspores generally germinate on the wall of the host cell, but it is not uncommon to find spores with long germ tubes in a drop of water quite apart from the host cell. Various stages in the germi-

nation of a spore and the development of the sporangium are shown in figures 2 to 11. These show successive stages of germination of a spore in a drop of water in which a bit of host tissue had been macerated.

The beginning of the germ tube is shown in figure 2 as a short papilla-like outgrowth on one side of the spore. Figure 3 shows the same spore twenty minutes later. The germ tube is comparatively thick and blunt at the end. In figure 4, showing the same spore forty minutes later, the germ tube is considerably longer, less in diameter, and more pointed at its tip than in the preceding figure. The cytoplasm of the spore appears more vacuolated in figures 3 and 4 than it was at the time germination began. Figures 5 and 6 show the spore seventy and ninety minutes after the beginning of germination. In figure 6 the end of the germ tube is beginning to enlarge, but as is shown in figures 7 and 8 the germ tube may still continue to elongate. In figure 6 the cytoplasm of the spore is highly vacuolated and the refractive body has moved from its previous position in the spore. In figure 7 it lies in the enlarged end of the germ tube. Its passage down the tube was quite slow as if it were being carried passively along in the moving cytoplasm. Figures 7 and 8 show the spore and germ tube two and three hours, respectively, after the beginning of germination. In both of these figures the zoospore itself is empty, and the base of the germ tube has enlarged until in figure 8 it is greater in diameter than the zoospore. In figure 7 is shown the beginning of the rhizoids which are generally present on the periphery of the mature sporangium. They begin as pseudopod-like outgrowths from the enlarged base of the germ tube, and continue to grow in the same fashion as the germ tube itself. Figure 7 shows the beginning of two such rhizoids, and in figure 8 are shown four, one somewhat perpendicular to, and three in the plane of, focus. Furthermore, in figure 8 the enlarged base of the germ tube is almost spherical in shape, and the number of highly refractive bodies in the cytoplasm appears to have increased. The stage here shown may be regarded as a very young sporangium. The germination of the zoospore here described is identical with that found by Cienkowski and Zopf in *E. Confervae glomeratae* and *E. Cienkowskiana*. The time occupied in the germination of the zoospore shown in figures 2 to 8 of my preparations was considerably longer than that described by Dangeard, who reports that germination takes place in thirty minutes. Cienkowski found that in *E. Confervae glomeratae* the spore penetrates the host cell and begins to form a sporangium in from one-half to two hours. It is to be noted, however, that the germ tube produced by the spore shown in figures 2 to 8 of my preparations is unusually long, and considerable time was occupied by its growth.

Unless the zoospores germinate shortly after coming to rest they die and disintegrate. Various stages of disintegration are shown in figures 24 to 26 b. Figure 24 shows a zoospore which had come to rest with the flagellum still attached. Figure 25 shows the same spore one hour later. In

the meantime it has increased considerably in diameter, due perhaps to the imbibition of water, and the cytoplasm has become highly vacuolated. A later stage is shown in figure 26 *a* in which the spore is fully twice its normal diameter, and the cytoplasm has drawn away from the wall into an apparently coagulated mass around the refractive body. Figure 26 *b* shows the same spore after its wall had burst. The refractive body, flagellum and remnants of spore wall and cytoplasm are still present. The refractive body and the flagellum may persist together for a considerable length of time.

It is to be noted that the stages shown in figures 2 to 8 are of a spore which had germinated in a drop of water in which a piece of host tissue had been macerated. When the zoöspores germinate on and penetrate the wall of the host cell the germ tube produced is generally much shorter than that shown in these figures. The method by which the germ tube penetrates the wall is not as yet clear. Whether it is accomplished by softening and dissolution of the wall or by piercing or boring through has not been determined. In some cases the germ tube appears to become smaller in diameter as it enters the wall of the host cell, and then enlarges immediately after getting through, as is shown in figures 13, 18, and 19. Figures 31, 32, and 30 show the germination of a zoöspore which had come to rest on a portion of an old cell of *Nitella*. The short germ tube shown in figure 31 is thick and blunt. Figure 32 shows its appearance after it had come into contact with and had penetrated the wall of the host cell for a short distance. There is a marked decrease in diameter of the germ tube in the region where it entered and began to grow in the host cell. In the case of this zoöspore the germ tube did not grow completely through the cell wall, but after a short distance turned sharply and grew laterally between the layers of the walls. The same spore is shown in figure 30 three hours after the beginning of germination. The length of the germ tube is fully ten times the diameter of the spore, and it is drawn to a fine needle-like point at its tip. The appearance of this germ tube suggests that perhaps the germ tubes pierce instead of softening and dissolving the wall of the host cell.

Occasionally a few zoöspores may fail to escape from the sporangium, and in such cases they germinate within the sporangium, as is well illustrated in figure 16. In *Rhizophidium brevipes* Atkinson (1909, fig. 2 *D* and *C*) reports a similar case in which several zoöspores germinated within and penetrated the wall of the sporangium. In the sporangium shown in figure 16 of my preparations are seven zoöspores which apparently failed to escape, four of which have germinated. The germ tubes of three of these spores have penetrated the wall of the sporangium and are in the process of forming sporangia themselves. I was fortunate in being able to keep these alive for two days, and saw two of the sporangia come to maturity. This figure indicates that sporangia may be formed without the zoöspores having first escaped to the outside of the host.

Zoöspores may occasionally germinate and produce sporangia without

the development of a conspicuous germ tube. This is well illustrated in figures 27, 28, and 29. Figure 27 shows a zoöspore which had germinated within a sporangium, and has begun to form a young sporangium itself without the production of a long germ tube. I was able to follow the successive stages of development of this sporangium up to the size shown in figures 28 and 29.

DEVELOPMENT OF THE SPORANGIUM

As shown in figures 6 and 7, the sporangium begins as an enlargement of the tip of the germ tube after it has penetrated the wall of the host cell. It appears to be formed very close to the inner boundary of the wall and in the primordial utricle of the host cell. Whether or not the sporangium may sometimes lie in the large central vacuole, I have not as yet determined. Figures 9, 10, and 11 show further successive stages of development of the young sporangium shown in figures 6 and 7. Figure 9 shows the young sporangium eight hours after germination of the spore. The spore and germ tube are entirely empty of cytoplasm, and although cut off from the sporangium by a wall, they still persist. When the zoöspores germinate on the host cell the old spore and germ tube generally disappear very soon, so that at the time the sporangium is of the size shown in figure 9 they are not to be found. Two exceptional cases, however, are shown in figures 13 and 14 in which the old zoöspore and germ tube are still present, although the sporangia are already mature. Furthermore, in figure 9 the rhizoids are considerably longer than in the preceding figure, and two of them have branched. The sporangium itself has become spherical in shape, and the number of refractive bodies in the cytoplasm has increased.

In figure 10 is shown the same sporangium six hours later. The old zoöspore and germ tube have begun to collapse and disintegrate, and the rhizoids have grown and branched further. The number of rhizoids produced on the surface of the sporangia varies. On some sporangia, as is shown in figures 11 and 13, I have observed only three and four, while on others I have found as many as twelve. These rhizoids generally consist of a main trunk from which are developed numerous small and fine branches which extend through long distances in the host cell. Although they are similar in appearance and habit of growth and branching to the mycelium of the higher fungi, it is questionable whether they should be regarded as such. They undoubtedly serve as haustoria in nourishing the developing sporangium. From the study of living material I have not thus far found any septation of the rhizoids.

Figure 11 shows the same sporangium twenty-four hours later. It is enveloped by a comparatively thick wall which cuts off the radiating rhizoids. The number of refractive bodies in the cytoplasm has increased, and the cytoplasm has perhaps a more granular appearance. On the surface of the sporangium may be seen an early stage in the development of the sporangial neck or tube through which the zoöspores will subsequently

escape when mature. Unfortunately this sporangium failed to develop further, and I was unable to follow its complete development through to the production of zoöspores.

An unusual case of the development of two sporangia from a single zoöspore is shown in figures 18 and 19. These show stages in the development of sporangia from a zoöspore which had germinated on a very small and thin piece of host tissue lying in a drop of water. A slight constriction of the germ tube is visible in the region where it penetrated the host tissue. Immediately after passing through, it enlarged at the tip and began to form a sporangium. Four hours later a small germ tube grew out from the base of the young sporangium, and after growing for a short distance in the water it enlarged at its tip and began to form another sporangium. Figure 19 shows the two sporangia eight hours later in a more advanced stage of development. I was able to follow the development of both sporangia through to maturity. This figure resembles to a certain degree the figure of *E. Cienkowskiana* by Zopf (1884, Pl. 6, fig. 17) in which he shows a fairly large sporangium with two smaller ones at its base. Such sporangia as are shown in figure 19 for *E. heliomorpha* are very rare.

STRUCTURE OF THE MATURE SPORANGIUM AND THE PRODUCTION OF ZOÖSPORES

The mature sporangium, as was described by Dangeard, is more or less oval or spherical in shape and varies considerably in size. The diameter is generally greater in the axis of the neck or tube. According to Dangeard and Fischer it varies from 10 to 20 microns. I have found some sporangia, such as are shown in figures 13 and 14, which were only 6 and 8 microns, while others were as much as 40 microns in diameter. The variation in size of the sporangia in the cells of various species of Characeae is shown in table 1. The cytoplasm is somewhat granular, with a large number of refractive bodies suspended or imbedded in it. Nuclei are generally invisible in living and unfixed sporangia.

Cleavage of the protoplast into spores appears to begin about the time the sporangial neck begins to penetrate the wall of the host cell, although it is not uncommon to find sporangia in which the sporangial neck is fully formed and has penetrated through the host wall before cleavage begins. Generally one refractive body is included in each cleavage segment of the protoplast. Figure 12 shows a large sporangium in which the process of cleavage is complete, and the individual zoöspores have been delimited. Due to the mutual pressure of the zoöspores on each other, they are generally hexagonal in shape at this stage. In this sporangium the neck has almost penetrated through the wall of the host cell, and appears to be filled with a somewhat granular liquid or cytoplasm. Usually only one tube or neck is formed for the escape of the zoöspores, but several sporangia with two and three necks have been observed.

The sporangial neck generally grows out for a short distance beyond the wall of the host cell before it ruptures at the apex. As it bursts, the liquid or cytoplasm in the tube rushes out. The zoöspores in the sporangium at this time become active, and almost immediately after the rupture of the sporangial neck they swim out. Their escape from the sporangium appears to be due to their own movement and not to excessive turgor in the sporangium. The zoöspores follow each other very closely, so that several may be found in the sporangial neck at one time, as is shown in figure 15. As they pass through the narrow neck they become very much elongated, but when once out they round up. The flagellum is drawn out behind the spore body in the fashion that is so characteristic of most of the Rhizidiaceae. Figure 15 shows a sporangium which contained forty-three zoöspores, all of which escaped in eighteen minutes. Several sporangia have been found with as many as sixty zoöspores. In this figure are shown three spores in the sporangial neck, and four which have escaped but whose flagella are apparently held in the neck by the closely crowding spores. Under such conditions the zoöspores appear to pull and strain from side to side in attempting to free themselves. They may also elongate, thrust out pseudopodia in several directions, and undergo various contortions to get free. When once free they immediately assume their spherical shape and dart away.

Two unusually small sporangia are shown in figures 13 and 14. These are the only two cases in which I have observed the old zoöspore and the germ tube persisting in the host cell after the sporangia were mature. The sporangium shown in figure 13 contained two zoöspores, while the one shown in figure 14 bore only one. Moreover, in this figure the sporangial neck is growing back up through the old germ tube. Such cases, however, appear to be unusually rare. After the zoöspores have escaped, the empty sporangium generally persists in the host cell until it disintegrates. It is not uncommon to find an old cell of *Nitella* or *Chara* full of empty and collapsed sporangia such as the one shown in figure 17.

DEVELOPMENT OF THE RESTING SPORE

The process of development of the resting spore is the same as that of the sporangium described above. An enlargement or sporangium is formed at the tip of the germ tube, but instead of undergoing cleavage into zoöspores and developing a sporangial neck, it becomes invested with a fairly thick wall. Figure 33 shows what appears to be a young resting spore. The wall is fully twice as thick as that of the mature sporangium. In the cytoplasm of the spore are a few refractive bodies of unequal sizes. These bodies often appear to run together in the process of maturing to form larger ones as is shown in figure 34. The spore shown in figure 33 has four rhizoid trunks, while those shown in figures 34 and 35 have two and one, respectively. As is shown in these figures, the resting spores are generally oval or spherical in

shape and vary in diameter from 8 to 24 microns. The formation of resting spores so far as has yet been observed appears to take place without any sexual fusion of cells.

I have not observed all stages in the germination of the resting spore. In figure 35, however, is shown a mature spore whose protoplast has undergone cleavage into spores. It is to be noted, however, that no sporangial neck is present. Although this spore was kept under observation for a considerable length of time no further development occurred, and the whole structure finally disintegrated. So far no one has figured the germination stages of the resting spores of *Entophlyctis*, although Zopf states in his description of *E. Cienkowskiana* that germination of the resting spore is similar to that of the sporangium. A number of resting spores are at present being kept under observation to determine the mode of germination.

HOSTS AND PATHOGENICITY OF ENTOPHLYCTIS HELIOMORPHA

According to Dangeard this chytrid occurs in cells of *Nitella*, *Chara*, and *Vaucheria*. I have found it in a large number of species of Characeae, including *Chara fragilis*, *C. delicatula*, *C. coronata*, *C. zeylanica*, *C. disjuncta*, *Nitella flexilis*, *N. gracilis*, *N. tenuissima*, and *N. glomerulifera*. Although the parasites appear more or less identical in their morphological characters on all of these hosts, it is not yet certain whether or not these forms growing on different hosts all belong to the same species. So far, physiological or biological races which are specific for certain host species have not been reported in this group of Chytridiales. With the view of comparing the size relations of the morphological characters which have been used as the basis of classification in the genus *Entophlyctis*, I have measured and tabulated the sizes of the zoöspores, sporangia, and resting spores of *E. heliomorpha* as they occur in the cells of three different species of Characeae.

TABLE I. Variation in *E. heliomorpha*, in Size of its Sporangia, Zoöspores, and Resting Spores, in Number of Rhizoids on Resting Spores, and in Character of the Wall of the Resting Spore, in the Cells of Three Species of Characeae

Host	Diameter of Sporangia	Diameter of Zoöspores	Diameter of Resting Spores	No. of Rhizoids on Resting Spores	Character of Wall of Resting Spore
<i>C. coronata</i>	7 x 7 μ —14 x 16 μ	3—4 μ	8 x 8 μ —14 x 16 μ	2—6	Smooth
<i>N. flexilis</i>	7 x 8 μ —16 x 18 μ	3—4 μ	10 x 12 μ — 14 x 16 μ	3—5	Smooth
<i>N. glomerulifera</i>	16 x 18 μ — 28 x 30 μ	3 μ	12 x 14 μ — 20 x 24 μ	2—7	Smooth

These measurements show the range in variation in the size of a hundred sporangia, zoöspores, and resting spores each. The diameters of these structures are on the average about the same in the cells of *C. coronata* and *N. flexilis*, but in *N. glomerulifera* they are considerably larger. In the cells of

this species of *Nitella* a few sporangia were twice as large as those found in *C. coronata*. The diameter of the zoöspores, however, is the same, and there is less variation in the size of the resting spores. The wall of the resting spores in all cases is smooth, and the variation in the number of rhizoids present on its surface is approximately the same. Although the sporangia, as noted, are unusually large in the cells of *N. glomerulifera*, this, it seems to me, is not sufficient ground for making the form on this host a new species of *Entophlyctis*.

A comparison of *E. heliomorpha* with other species of this genus shows considerable similarity in size and morphological structure. This comparison can be well presented in tabular form, as is shown in table 2. In 1896 Wildeman described and figured the resting spores of an *Entophlyctis* in oogonia of *Chara* which he called *E. Characearum*. The presence of one and rarely two rhizoids localized at the base of the resting spore is the distinguishing characteristic of this species, according to Wildeman. It is to be noted in this connection that in *E. Confervae glomeratae* and *E. heliomorpha* resting spores with only one rhizoid may occasionally be found, and since Wildeman did not find sporangia and zoöspores it is not improbable that the resting spores which he discovered belonged to *E. heliomorpha*.

TABLE 2. *Size of Sporangia, Zoöspores, and Resting Spores, Number of Rhizoids on Resting Spores, and Character of Spore Wall, in Seven Species of Entophlyctis*

Host	Species	Sporangium	Zoöspores	Resting Spores	No. of Rhizoids on Resting Spore	Wall of Resting Spore
<i>Cladophora</i>	<i>E. Cienkowskiana</i> (Zopf) Fischer	5-25 μ	3-5 μ	5-26 μ	1-4	Smooth
Oogonia of <i>Chara</i>	<i>E. Characearum</i> Wild.	Unknown	Unknown	17-25 μ	1, rarely 2	Smooth
<i>Nitella</i> , <i>Chara</i> , <i>Vaucheria</i> , <i>Conferva</i>	<i>E. heliomorpha</i> (Dang.) Fischer	8-30 μ	3-4 μ	8-24 μ	2-7	Smooth
	<i>E. Confervae glomeratae</i> (Cienk.) Fischer	10-23 μ	3-5 μ	5-25 μ	2-3	Smooth
<i>Spirogyra</i>	<i>E. tetrasporium</i> (Sorok.) Fischer	Very small	Only four	Unknown	Unknown	Unknown
<i>Gleococcus</i>	<i>E. apiculata</i> (Braun) Fischer	11-13 μ	Very small	11-13 μ	—	—
<i>Spirogyra</i>	<i>E. bulligera</i> (Zopf) Fischer	25 μ	—	Unknown	2-5 μ	—

This table shows that with the exception of *E. tetrasporium* and *E. apiculata*, which according to Sorokine's (1883) and Braun's (1855) descriptions are very minute, the size of the sporangia, zoöspores, and resting spores, and the general habit of growth of all of these species of *Entophlyctis* are very much the same. Zopf and Fischer consider *E. Confervae glomeratae* and *E. Cienkowskiana* as one species on the basis of their similarity in size, method of development, and structure. The life history of *E. heliomorpha*

as described by Dangeard and myself is identical with that of these two species, and it differs from them only in its host relationship. No intensive study so far has been made to determine the range and number of hosts of any species of *Entophlyctis*, and it is perhaps not improbable that a single species may have a number of different host plants. An attempt is being made at present to infect with *E. heliomorpha* various algae on which *Entophlyctis* has been reported to occur, to determine the limit of its range of hosts.

Most species of *Entophlyctis* so far described appear to be saprophytic or at most weakly parasitic. Zopf and Fischer report that *E. Confervae glomeratae* and *E. Cienkowskiana* are generally found only in dead or partly dead host cells. As noted before, *E. heliomorpha* usually occurs in dead cells of the Characeae, and so far I have not discovered it in living green cells. However, the presence of the deep green chlorophyll in such cells may readily obscure the hyaline sporangia and zoöspores. A considerable number of other saprophytes and parasites, such as *Diplophlyctis intestina* (Schenk) Schroeter, *Diplophysalis stagnalis* Zopf, *D. Nitellarum* Cienk., *Vampyrella*, and protozoa, are generally associated with *E. heliomorpha* in dead cells of the Characeae.

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EXPLANATION OF PLATE I

All drawings were made from living material with the aid of a Spencer camera lucida, a Zeiss 2 mm. apochromatic objective N.A. 1.30, and compensating ocular number 8. Approximate magnification 1,800.

FIG. 1. A mature zoospore with nucleus, refractive body, and long flagellum.

FIG. 2. A zoospore just beginning to germinate (Figures 2-11 show successive stages in the germination of a zoospore in a drop of water, and the formation of the sporangium).

FIG. 3. The same spore twenty minutes after the beginning of germination.

FIG. 4. Forty minutes after germination.

FIG. 5. Seventy minutes after germination.

FIG. 6. Ninety minutes after germination. The tip of the germ tube is beginning to enlarge.

FIG. 7. Two hours after the beginning of germination. The refractive body lies in the enlarged tip of the germ tube. The rhizoids are beginning to develop as small pseudopods.

FIG. 8. Three hours after germination. The spore is empty of cytoplasm, and the number of rhizoids has increased.

FIG. 9. Eight hours after germination. The spore and germ tube are empty, and the young sporangium has become enveloped by a thin wall. Two of the rhizoids have begun to branch.

FIG. 10. Fourteen hours after the beginning of germination.

FIG. 11. Twenty-four hours after germination. The spore and germ tube have collapsed, and the sporangial neck is beginning to develop on the surface of the sporangium.

FIG. 12. A mature sporangium which has undergone cleavage into zoospores. The sporangial neck has almost penetrated the wall of the host cell.

FIG. 13. A small mature sporangium with two zoospores. The old spore and germ tube are still present.

FIG. 14. A small sporangium with a single zoospore. The sporangial neck has grown up through the old germ tube.

FIG. 15. A mature sporangium with escaping zoospores.

FIG. 16. Zoospores germinating within the sporangium.

FIG. 17. An empty collapsed sporangium.

FIGS. 18, 19. Stages in the development of two sporangia from a single zoospore.

FIGS. 20-23. Changes in the shape of a zoospore in attempting to free itself from a tight position between the slide and cover glass.

FIGS. 24-26. Stages in the disintegration of a zoospore.

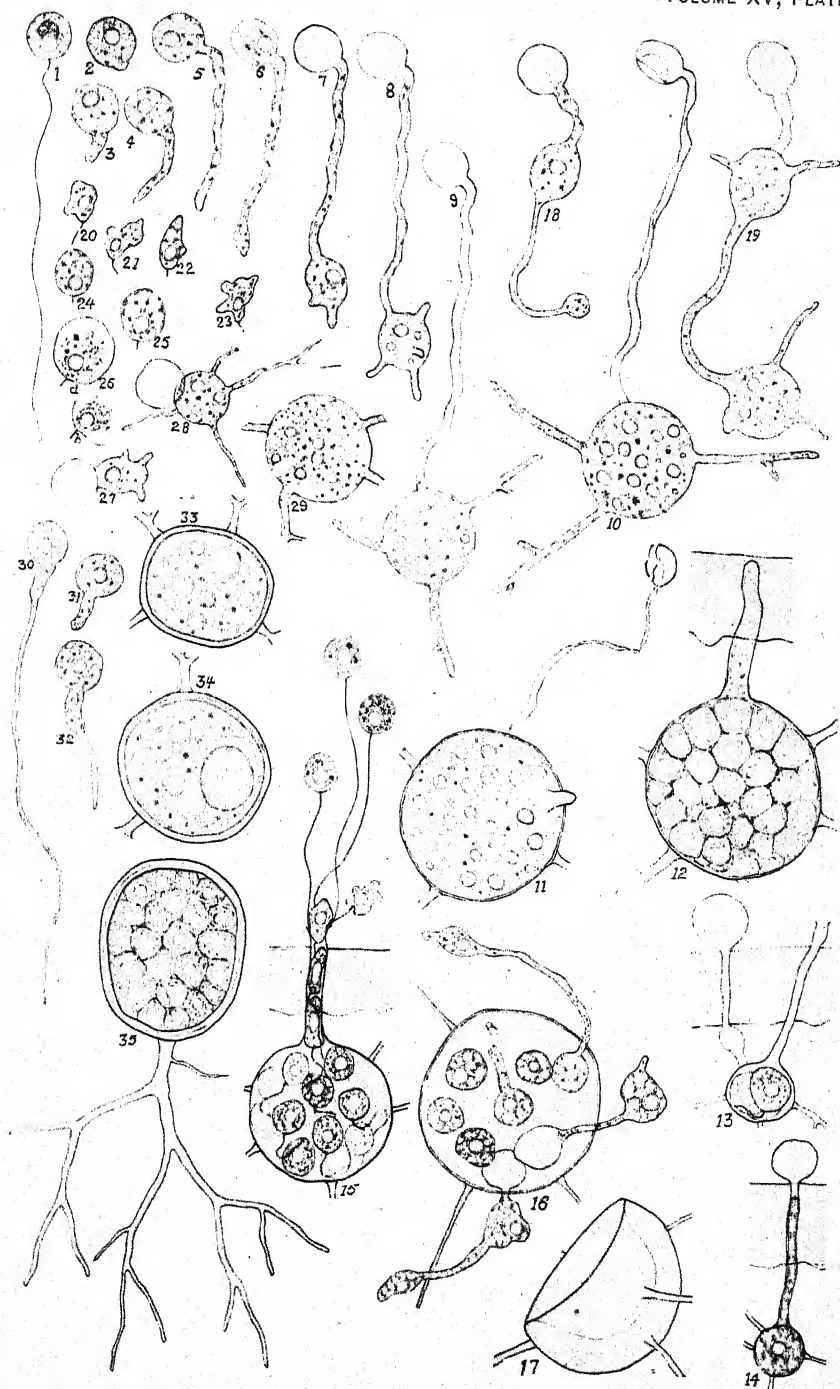
FIGS. 27-29. Stages in the development of a sporangium without the production of a long germ tube.

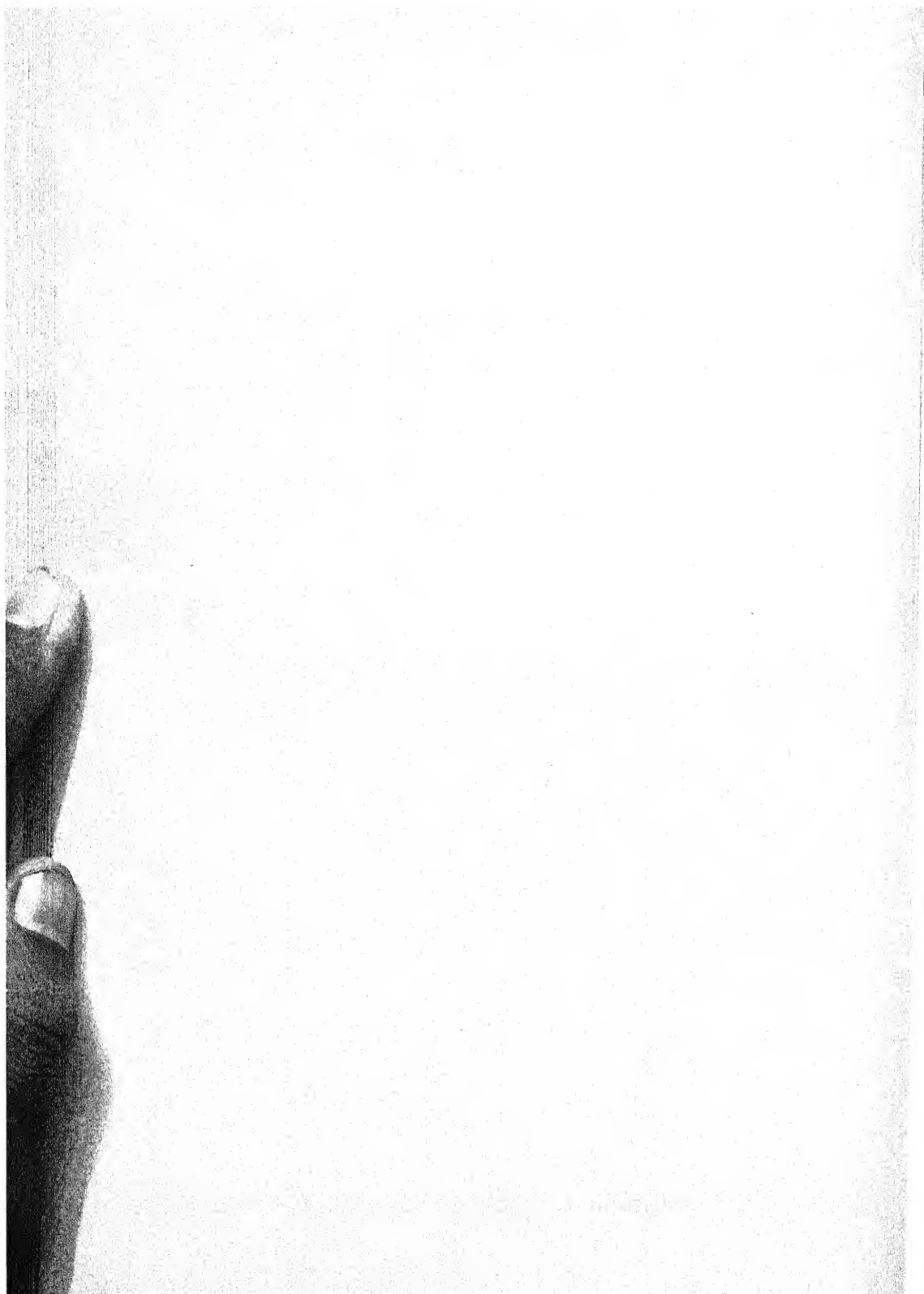
FIGS. 30-32. Stages in the germination of a zoospore.

FIG. 33. A young resting spore with numerous refractive bodies of various sizes.

FIG. 34. Perhaps an older resting spore with two rhizoid trunks.

FIG. 35. A mature resting spore with one rhizoid trunk which has undergone cleavage.





REVIEW OF THE GENUS *DIPLOSTEPHIUM*

S. F. BLAKE

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In 1922 the writer¹ published a revision of *Diplostephium*, a genus closely allied to *Aster* but distinguished by habit and by its double pappus. Forty species were recognized, in addition to one of uncertain position, divided into five series on characters of inflorescence and foliage. In consequence of the fact that a third of the species were known to me only by description, one or two were misidentified, and at least three were redescribed under new names. The receipt, through the kindness of Dr. L. Diels and Dr. J. Mattfeld, of a set of fragments of all the species described by Hieronymus, and the opportunity to examine various other types in European herbaria in 1925, have made it possible to rectify most of the errors of the earlier treatment and to present a new review of the genus.

The taxonomic history of the genus was sufficiently sketched in my earlier paper, but its distribution calls for more discussion than was there given it. *Diplostephium* now consists of 43 species, with their center of abundance in Colombia, where 24 species are found; 11 or 12 are found in Ecuador, 10 or 11 in Peru, 2 in Chile, and 1 each in Costa Rica, Venezuela, and Bolivia. One species, *D. ochraceum*, is of doubtful habitat, but is probably either Colombian or Ecuadorian. All the species are nearly or quite confined to the páramo or its lower bordering shrub zone, the páramillo, usually at altitudes of 3000 to 4270 meters, or perhaps even higher. Several species occur down to 1830 meters in Peru, and *D. costaricense* was collected by Pittier in humid woods at La Palma, Costa Rica, altitude 1500 meters, but also occurs in páramo thickets at 2700 to 3000 meters.

The local distribution of the 24 species definitely known from Colombia, although as yet imperfectly known, offers some points of interest. Four species only (*D. anactinotum*, *D. parvifolium*, *D. cyparissias*, and *D. weddellii*) are known from the Sierra Nevada of Santa Marta, where all of them are endemic. At least two species (*D. schultzei* and *D. floribundum*) occur in both the central and western cordilleras, and one, *D. baccharideum*, is found in both the eastern and western cordilleras, even to the extreme northern limit of the latter, but is not known from the central cordillera.

The genus may be regarded as an Andean development of *Aster*, which has taken on the shrub habit and further adapted itself to the extreme conditions prevailing on the páramos by the development of tomentum on the branches and under surface of the leaves. Specific differences are furnished chiefly by the character of the inflorescence, the shape, size, and

¹ Contr. U. S. Nat. Herb. 24: 65-86, pls. 21-28. 1922.

pubescence of the leaves, and the size of the involucre and heads. Of the five series distinguished in my earlier paper, the three last (*Rupestris*, *Floribunda*, and *Denticulata*) are here combined under the name *Rupestris*. The characters relied on in separating them are often uncertain, and the presence or absence of regular dentation in the leaves is not even of specific value.

The most interesting feature of the morphology of *Diplostegium* is found in the variation exhibited by the styles of the disk flowers. In the earlier revision three principal types of styles were distinguished and the representative species listed. Many of the species not seen at that time have since been examined, and a new list can now be presented. The style characters in *D. jelskii*, *D. cochense*, *D. obtusum*, and *D. ochraceum* are still unknown or insufficiently described, as well as those in authentic material of *D. meyenii*, but the last species is not likely to differ from *D. tacorense*. The specific names in quotation marks in the following lists are included on the basis of Weddell's descriptions.

1. Style more or less clavate, merely bifid or even subentire, the branches very short, ovate, usually obtuse, merely papillose: *D. micradenium* (Series *Lavandulifolia*); *D. revolutum*, *D. baccharideum*, *D. rosmarinifolium* (Series *Rosmarinifolia*); *D. rupestre*, *D. eriophorum*, *D. schultzei*, *D. costaricense*, *D. lehmannianum*, *D. rhododendroides* (approaching type 2), *D. pleistogynum*, *D. floribundum* (Series *Rupestris*).

2. Style branches of medium length, oblong, linear-oblong, or lanceolate, acute, merely papillose: *D. anactinotum*, "*D. parvifolium*" (Series *Lavandulifolia*); "*D. cyparissias*" (Series *Rosmarinifolia*). A style intermediate between type 2 and type 1 is shown by *D. phyllioides* (Series *Rupestris*), in which the branches are linear, acute, and hispidulous dorsally, but often connivent nearly to apex; and styles more or less intermediate between types 2 and 3 are found in *D. weddellii*, *D. tacorense*, *D. leiocladum*, and *D. denticulatum* (Series *Rupestris*).

3. Style branches elongate, linear-subulate, acuminate, hispidulous or hispid dorsally, the appendage sometimes thrice as long as the stigmatic region: *D. empetrifolium*, *D. glandulosum*, *D. oblanceolatum*, *D. macrocephalum*, *D. pearcei*, *D. callilepis*, *D. hippophae*, *D. adenochaenium*, *D. gnidioides*, *D. lavandulifolium*, *D. hartwegii* (Series *Lavandulifolia*); *D. carabayense* (Series *Rosmarinifolia*); *D. lechleri*, *D. pulchrum*, *D. haenkei*, *D. bicolor* (Series *Rupestris*). In *D. spinulosum* and *D. antisanense* (Series *Lavandulifolia*) the form of the style branches is much the same, but they are merely papillose, and in *D. spinulosum* appear to lack stigmatic lines.

In my earlier paper it was suggested that the primitive type of style in *Diplostegium* is probably type 2, which approximates most nearly the average or mean type of the tribe, and that types 1 and 3 have been derived from it. It is equally possible that type 3, a form by no means uncommon in the *Astereae*, is primitive, and that type 1 and type 2 have been derived from it. In either case, the evolutionary course of the genus is apparently directed toward the development of two groups, one with subentire or slightly bifid styles and sterile disk flowers, the other with long-appendaged styles and fertile disk flowers. This evolutionary tendency does not align with the division into three series here adopted on the basis of inflorescence and foliage—a division which, taking all the characters of the plants into

consideration, seems to be a highly natural one—but cuts across it. Most of the species of the Series *Lavandulifolia* fall into type 3, but *D. micradentum* is typical of type 1, and one or two other species enter type 2. Three of the five species of Series *Rosmarinifolia* are of type 1, one is of type 3, and one, from description, appears to belong to type 2. Eight species of the Series *Rupestris* belong to type 1, four are of type 3, and five are variously intermediate. That is, each of the two groups which may eventually attain generic distinction by the development of differences in style and concomitant fertility or sterility of disk flowers is composed of species from each of the three apparently very natural series into which the genus can now be divided.

In connection with the evolutionary possibilities here discussed, the variation shown by *Diplostephium schultzei* is of special significance. This species is one of those with barely bifid style and a tendency to sterility of the disk. In two collections examined, including the type number, the heads were heterogamous, with 10 to 44 rays and 4 to 32 disk flowers, a variation in itself far beyond that known in any other species. Four heads dissected from another collection, certainly referable to the same species, were homogamous, with 27 to 38 ligules and no hermaphrodite flowers whatever. The individual plant or plants from which these specimens were collected apparently represent a remarkable mutation, of a sort I have never before met with in wild Asteraceae, in which the head is composed wholly of pistillate ray flowers. Whether a corresponding mutation resulting in the production of discoid heads composed of staminate (infertile hermaphrodite) flowers has taken place is uncertain, and probably unlikely, but it is evident that a careful field study of this species would be of the highest interest. The abortion of the ovules, in the achenes examined, would indicate that no pollen was available in the vicinity of the plant or plants from which these specimens were obtained.

The following abbreviations are used in this paper to refer to herbaria in which specimens are deposited: F = Field Museum; G = Gray Herbarium; K = Kew Herbarium; N = U. S. National Herbarium; Par. = Herbarium of the Museum d'Histoire Naturelle, Paris. Except in a few cases, where corrections in identification have been made, specimens already cited in my previous paper have not been listed. My thanks are due the curators of the herbaria mentioned for the opportunity to study and borrow the material under their charge.

KEY TO SERIES

Heads solitary at tips of stem and branches.....1. *Lavandulifolia*.
Heads cymose or cymose-panicled.

Leaves linear or essentially so, 2 mm. wide or less.....2. *Rosmarinifolia*.

Leaves linear-lanceolate to oblong or obovate, very rarely linear, 3-30 mm. wide.

3. *Rupestris*.

KEY TO SPECIES

1. *Lavandulifolia*

Leaves densely and conspicuously impressed-glandular over whole upper surface.

Leaves finely impressed-glandular above, linear, about 25 mm. long, 1.5-2 mm. wide, densely tomentose-pilose beneath and when young also sparsely pilose on upper surface with comparatively long, loose, not crisped hairs; branches densely pilose-tomentose with reflexed hairs. 1. *D. micradenium*.

Leaves coarsely impressed-glandular above, their tomentum dense, without long loose hairs; branches closely tomentose, in one species glabrate or glabrescent.

Leaves 4-7 mm. long, essentially linear, not spinulose above; involucre 7-9 mm. high. 2. *D. empetrifolium*.

Leaves usually 10-22 mm. long, if smaller then spinulose above; involucre 10-13 mm. high.

Larger leaves narrowly oblanceolate or linear-spatulate to nearly linear, 3 mm. wide or less, closely ochraceous- or cinereous-tomentose beneath, the costa glabrate and evident at least toward base; branches closely cinereous- or ochroleucous-tomentose, usually glabrate or glabrescent; heads short-peduncled. 3. *D. glandulosum*.

Larger leaves oblanceolate or spatulate-oblanceolate, 15-22 mm. long, 3.5-4.5 mm. wide, densely ochraceous- or fuscous-lanate beneath, the costa hidden; branches densely brownish-lanate-tomentose; heads sessile or subsessile.

4. *D. oblanceolatum*.

Leaves not at all impressed-glandular above or only obscurely so (in *D. tacorense* and *D. meyenii*), sometimes spinulose, glandular-tuberculate, or glandular.

Involucre 10-15 mm. high.

Leaves narrowly spatulate or oblanceolate-spatulate, obtuse, fuscuscently lanate-tomentose beneath, glandular-tuberculate above. 5. *D. macrocephalum*.

Leaves strictly linear, acute or acutish, not glandular-tuberculate above.

Branches densely tomentose, not setulose.

Leaves spinulose along margin above; achenes glabrous or sparsely glandular.

6. *D. pearcei*.

Leaves glabrous above; achenes hirsutulous and glandular. 7. *D. callilepis*.

Branches closely whitish-tomentose and densely setulose with thick yellowish hairs; leaves spinulose along costa above, often also along margin; achenes sparsely hirsutulous and toward apex glandular. 8. *D. hippophae*.

Involucre 4-9 mm. high.

Leaves densely glandular or resinous-coated but not pubescent above.

Leaf blades elliptic or oval, broadly rounded, definitely petioled.

9. *D. anactinotum*.

Leaf blades triangular-oblong, acutish or obtuse, sessile. 10. *D. parvifolium*.

Leaves not densely glandular or resinous-coated above, either glabrous, pubescent, glandular-tomentose, or spinulose.

Leaf blades oblong or ovate-oblong, about 8 mm. long, 2.5 mm. wide, blackish-lanate-tomentose beneath, appearing sessile, the angled petioles about 1.5 mm. long, closely appressed to the stem and appearing adnate to it; involucre black-lanate-tomentose. 11. *D. spinulosum*.

Leaf blades linear to elliptic, or if tending toward oblong then with distinctly spreading petioles; involucre not black-tomentose.

Leaf blades elliptic to linear-oblong, 7-11 mm. long, 1.5-3 mm. wide, soon glabrous and shining above, not spinulose. 12. *D. adenochaenium*.

Leaf blades linear to narrowly elliptic, not over 2 mm. wide.

Leaf blades glandular-tomentose above at least when young, narrowly linear or linear-oblongate, 8-30 mm. long.....13. *D. meyenii*.
14. *D. iacorensis*.

Leaf blades sometimes tomentose but not glandular above, 3.5-11 mm. long.

Leaves acutely callous-pointed, the tomentum of the lower surface usually concealed by the strongly revolute margin.

Leaves glabrous above; achenes densely hirsutulous.

15. *D. jelskii*.

Leaves spinulose above; achenes very sparsely hirsutulous.

16. *D. gnidioides*.

Leaves obtuse, or if callous-pointed (*D. hartwegii*) then with tomentum of lower leaf surface conspicuous.

Involucre 6-7 mm. high.

Stem and leaves beneath densely appressed-tomentose with whitish or subcanescent hairs.....17. *D. lavandulifolium*.

Stem and leaves beneath densely and loosely tomentose with ochroleucous or reddish hairs.....18. *D. hartwegii*.

Involucre 4-5 mm. high.....19. *D. antisanense*.

2. *Rosmarinifolia*

Branchlets glabrous, more or less viscid.....20. *D. revolutum*.
Branchlets densely tomentose, sometimes glabrescent.

Heads comparatively large, the involucre about 9-10 mm. high; leaves 8-15 mm. long, 1.5-2 mm. wide.....21. *D. carabayense*.

Heads smaller, the involucre 5-7 mm. high; leaves (0.9) 1.5-4.2 cm. long.

Leaves slightly revolute-margined, pale green and persistently viscid-tomentellous above, strictly sessile; heads subsessile.....22. *D. cyparissias*.

Leaves strongly revolute-margined, dark green and glabrate or glabrescent above, shortly but definitely petioled; heads pedicellate (pedicels usually 2-8 mm. long).

Leaves 1-2.5 cm. long; disk achenes sparsely hispid.....23. *D. baccharideum*.

Leaves 2.7-4.2 cm. long; disk achenes glabrous.....24. *D. rosmarinifolium*.

3. *Rupestris*

Heads large, mostly on monocephalous peduncles in a usually simple or subsimple leafy-bracted cyme; involucre (8) 9-12 mm. high.

Leaves sessile by a comparatively broad base, impressed-punctate above (sometimes obscurely so in *D. weddellii*).

Branchlets closely ochraceous-tomentose; heads subsessile.....25. *D. weddellii*.

Branchlets densely lanate-tomentose or pilose with spreading hairs; heads distinctly peduncled.

Branchlets pilose or pilose-tomentose; leaves strongly viscid above, mostly linear or linear-elliptic (by revolution of the margin), 2.5-5 cm. long, 3-7 mm. wide.....26. *D. rupestre*.

Branchlets densely lanate-tomentose; leaves scarcely viscid above, elliptic or ovate-elliptic, 1.5-2.5 cm. long, 4-10 mm. wide.....27. *D. eriophorum*.

Leaves distinctly petioled or narrowed into a petioliform base, not impressed-punctate above.

Leaves white-sericeous or -subsericeous beneath with a very close and comparatively thin but persistent tomentum.

Leaves glabrous above, except on costa; branches closely and persistently ochraceous-tomentose.....28. *D. lechleri*.

Leaves hispidulous on surface above; branches densely hirsute-pilose with yellowish hairs as well as tomentose, the tomentum more or less deciduous.

29. *D. pulchrum*.

Leaves usually ochroleucous- to ferruginous-tomentose beneath, the tomentum dull, thick, and comparatively loose..... 30. *D. haenkei*.

Heads smaller, usually in a cymose panicle; involucre 4.5-7 (8) mm. high.

Leaves very small, elliptic or oblong, thick-coriaceous, 5-11 mm. long, 1.8-3 mm. wide, abruptly very short-petioled, not narrowed toward base of blade; heads in a close, essentially simple, umbelliform cyme..... 31. *D. phyllioides*.

Leaves larger, mostly obovate or oblanceolate, narrowed to a petiole or petioliform base; heads usually cymose-panicled.

Leaves persistently gray-tomentose or -tomentulose above, sometimes glabrate at length (in *D. obtusum*).

Lateral veins 3-4 pairs, spreading at an angle of 45-60°, practically concealed beneath by the tomentum.

Heads 27-50-flowered; larger leaves 2-3.3 cm. long..... 32. *D. schultzei*.

Heads 12-15-flowered; larger leaves 2 cm. long..... 33. *D. cochense*.

Lateral veins 6-10 pairs, spreading at nearly a right angle, usually evident beneath.

Leaves acute or acutish, acutely callous-tipped; heads 22-57-flowered.

34. *D. costaricense*.

Leaves rounded or emarginate, not callous-apiculate; heads 18-21-flowered.

35. *D. obtusum*.

Leaves green and glabrous or quickly glabrate above (except sometimes on costa).

Larger leaves 1.5-2.7 cm. long.

Involucre 4.5-5 mm. high; leaves 1.5 cm. long or less, obovate.

36. *D. lehmannianum*.

Involucre 7-8 mm. high; leaves 1.3-2.7 cm. long.

Leaves obovate, 2-2.5 cm. long, 8-10 mm. wide; rays 6-8, disk flowers about 20..... 37. *D. rhododendroides*.

Leaves oblanceolate, 1.3-2.7 cm. long, 4-7 mm. wide; rays about 27, disk flowers 7..... 38. *D. pleistogynum*.

Larger leaves 4-10 cm. long.

Lateral veins slender, straightish, spreading from the costa at nearly a right angle; petioles narrowly margined essentially to base.

Branchlets and peduncles persistently tomentose..... 39. *D. floribundum*.

Branchlets and peduncles quickly nearly or quite glabrate.

40. *D. leiocladum*.

Lateral veins stout, curved, spreading at an angle of about 45-60°; petioles naked.

Leaf blades pergamentaceous, the secondary veins conspicuous and reticulate beneath; achenes pubescent..... 41. *D. denticulatum*.

Leaf blades more or less coriaceous, not conspicuously reticulate beneath, the secondary veins mostly concealed by the tomentum; achenes glabrous or sparsely glandular.

Leaf blades elliptic-oblong, rounded or bluntly cuneate at base.

42. *D. ochraceum*.

Leaf blades obovate or oblong-obovate, acutely cuneate at base.

43. *D. bicolor*.

SERIES I. LAVANDULIFOLIA Blake, Contr. U. S. Nat. Herb. 24: 69. 1922.

Heads solitary at tips of stem and branches or branchlets, the latter often numerous and crowded.

1. *Diplostephium micradenium* Blake, sp. nov.

Branches densely ochroleucous-pilose-tomentose with straightish reflexed hairs; leaves linear, about 2.5 cm. long, obtuse, sessile, above green, impressed-punctate, when young sparsely and loosely pilose, beneath densely ochroleucous-pilose-tomentose with comparatively straight, antrorse hairs, strongly revolute-margined; heads solitary, large; involucre 1-1.2 cm. high.

Shrub; branches few, erect, dichotomous, smoothly denudate in age, 2-4 mm. thick, terete, their pubescence grayish in age; internodes 1.5-3 mm. long; leaves erectish, 2-3.2 cm. long, 1.5-2.2 mm. wide, obtusely callous-tipped, with broad costa; peduncle about 1 cm. long; disk about 12 mm. high; involucre strongly graduate, about 5-seriate, the phyllaries lance-ovate (outer) to linear, acute to subacuminate, whitish and indurate, with purplish-brown tips and the costa thickened and darkened above, loosely and sparsely pilose and ciliate, sessile-glandular; rays "white," the tube hirsutulous above, 3.5 mm. long, the lamina linear-elliptic, 4-nerved, about 10 mm. long, 1.8 mm. wide; disk corollas "brownish yellow," numerous, hirsutulous on throat, slender, about 6 mm. long (tube 1.5 mm., throat 3.5 mm., teeth 1-1.3 mm.); achenes 4-5-nerved, rather densely hirsutulous and toward apex sessile-glandular, those of the ray compressed, 4 mm. long, 2 mm. wide, of the disk linear, infertile, 5-6 mm. long; pappus bristles brownish, 7 mm. long, slightly enlarged above, the outer pappus rather obscure, setulose, 0.5-1.2 mm. long; style of disk flowers finely hirsutulous above, minutely bilobate.

COLOMBIA: Shrub zone, below páramo, Cerro Tatama, Cordillera Occidental, Dept. Caldas, alt. 3300-3500 m., 8-10 Sept. 1922, *Pennell* 10533 (type no. 1, 141, 318, U. S. Nat. Herb.).

A very distinct species, remarkable for the apparently complete sterility of the disk.

2. *DIPLOSTEPHIUM EMPETRIFOLIUM* Blake, Contr. U. S. Nat. Herb. 24: 73. *pl.* 21. 1922.

RANGE: Ecuador, Peru (?).

ADDITIONAL SPECIMENS EXAMINED: ECUADOR: Between San Lucas and Oña, Prov. Loja, alt. 2200-3100 m., 7 Sept. 1923, *Hitchcock* 21526 (N).

This species is not closely allied to any other. It is readily recognized by its very small, uniform, acutely callous-pointed leaves, green and densely impressed-glandular above, and closely tomentose beneath. The rays, in Hitchcock's comparatively fresh specimens, were evidently white; the disk corollas, at least in age, are purple.

3. *DIPLOSTEPHIUM GLANDULOSUM* Hieron. Bot. Jahrb. Engler 21: 339. 1895.

Diplostephium spinulosum Hieron. Bot. Jahrb. Engler 29: 22. 1900; Blake, Contr. U. S. Nat. Herb. 24: 76. 1922. Not *D. spinulosum* Wedd. (err. ident.).

Diplostephium cicatricosum Blake, Contr. U. S. Nat. Herb. 24: 76. *pl.* 23. 1922.

TYPE LOCALITY: Páramo de la Boca del Mundo Nuevo, Volcán de Cumbal, Colombia.

RANGE: Colombia, Ecuador.

ADDITIONAL SPECIMENS EXAMINED: COLOMBIA: Paramo de la Boca del Mundo Nuevo, Volcán de Cumbal, Jan.-Feb. 1870, *Stuebel* 445 b (type of *Diplostephium glandulosum*; fragm. N); same locality, *Karsten* (herb. Sch. Bip., fragm. N). Tuquerres, Prov. Tuquerres, alt. 3200 m., *Triana* 1257 (Par.). Mt. Azufra, Cordillera Meridional, 18 May 1876, *André* (G). ECUADOR: Pilzhun, alt. 3965 m., *Jameson* (G). Arid hills near Riobamba, Sept. 1893, *Sodiño* 64/3 (fragm. N). Mirador, July 1902, *Rivet* 183 (Par.), Aug. 1902, *Rivet* 194 (Par.). Naes, Feb. 1903, *Rivet* 358 (Par.). El Pelado, Jan. 1903, *Rivet* 312, 315, 333 (Par.). Quinoaloma, Mar. 1904, *Rivet* 590 (Par.).

In my 1922 revision, *Diplostephium glandulosum* Hieron. was doubtfully referred to *D. spinulosum* Wedd., the latter being interpreted by Hieronymus' ample description² based on *Sodiño* 64/3. Examination of the type collection of *D. spinulosum*, of fragments of two collections (including the type) of *D. glandulosum*, and of fragments of *Sodiño* 64/3 has now shown that *D. spinulosum* is a very distinct species and that *D. glandulosum* Hieron., *D. cicatricosum* Blake, and "*D. spinulosum*" of Hieronymus and of Blake, not of Weddell, are to be combined under the earliest name, *D. glandulosum*. The specimens cited under *D. spinulosum* in my previous paper belong to *D. glandulosum*. Karsten's plant in the Schultz Bipontinus herbarium bears unpublished specific and varietal names of Schultz.

Rivet's labels describe the flowers as rose, violet, white, or yellowish. The last can scarcely refer to the color of the rays. The plant is a shrub up to about 45 cm. high, usually fasciculately branched above.

4. DIPLOSTEPHIUM OBLANCEOLATUM Blake, Contr. U. S. Nat. Herb. 24: 76. pl. 24. 1922.

RANGE: Ecuador.

ADDITIONAL SPECIMEN EXAMINED: ECUADOR: Minas, Nov. 1904, alt. 4000 m., Nov. 1904, *Rivet* 735 (Par., fragm. N).

This species is close to *D. glandulosum*, differing chiefly in its densely and persistently tomentose branchlets, usually larger leaves with the costa concealed beneath, and sessile or subsessile heads, those of *D. glandulosum* being normally borne on short but evident peduncles 5-15 mm. long. Rivet notes the color of the flowers as violet.

5. DIPLOSTEPHIUM MACROCEPHALUM Blake, Contr. U. S. Nat. Herb. 24: 75. pl. 22. 1922.

RANGE: Ecuador.

ADDITIONAL SPECIMENS EXAMINED: ECUADOR: Niarihuina, alt. 3900 m., Dec. 1904, *Rivet* 738, 743 (Par.).

Closely related to *D. oblanceolatum*, and distinguished principally by the

² Bot. Jahrb. Engler 29: 22. 1900.

entire lack of impressed glands on the leaves, which are conspicuously tuberculate with blunt papillae above toward margin and tip. The flowers are described as violet by Rivet. The plant is a low shrub under 30 cm. high.

6. *Diplostephium pearcei* Sprague & Blake, sp. nov.

Branches densely griseous-tomentose; leaves linear, 7-11 mm. long, 1-1.5 mm. wide, acute, green above, spinulose along margin and at apex, densely griseous-tomentose or in age fuscous-tomentose beneath, strongly revolute-margined; heads solitary, large; involucre 12-15 mm. high; achenes glabrous or sparsely glandular.

Shrub 0.6-1.3 m. high, fastigate-branched, very leafy; stem glabrate, terete, 6 mm. thick, the extreme bases of the leaves persistent, appressed; tomentum of the branches becoming fuscous; leaves spreading, obscurely short-petioled, not glandular-punctate, the costa impressed above, concealed beneath; heads sessile at tips of branches, 2.5-3 cm. wide; involucre hemispheric, 1.2-1.5 cm. high, about 6-seriate, strongly graduate, the phyllaries lance-ovate (outermost) to linear, acute or subacuminate, pale and indurate with narrow thin lacerate-ciliate margin, the outer persistently fuscous-tomentose dorsally, the inner glabrous or glabrate dorsally; rays "white," 36 or more, the tube 1.5 mm. long, sparsely hirsutulous, the lamina linear, 3-denticulate, 13.5 mm. long, 1.5 mm. wide, 4-nerved; disk corollas numerous, sparsely hirsutulous toward base of throat, 5.3 mm. long (tube 1.5 mm., throat 3 mm., teeth 0.8 mm.); ray achenes (immature) 1.5 mm. long, obscurely glandular above or essentially glabrous, their pappus brownish white, the inner bristles 5.5 mm. long, the outer 3.5 mm.; disk achenes (immature) sparsely glandular, 1.5 mm. long, their pappus brownish white, the inner bristles 5.5 mm. long, the outer of aristiform squamellae about 1 mm. long, sometimes aristate-tipped; style appendages subulate-acuminate, evenly hispidulous, 1.3 mm. long.

PERU: Boggy places in hills, Puitac (near Huanta), alt. 3660-3965 m. February 1867, *R. Pearce* (types in Kew Herbarium and Brit. Mus.; photog. and fragm., U. S. Nat. Herb.).

Allied to the Ecuadorian *Diplostephium macrocephalum* Blake, which is readily distinguished by its narrowly oblanceolate or spatulate-oblanceolate, obtuse, glandular-tuberculate leaves, 1.5-3 mm. wide.

7. *Diplostephium callilepis* Blake, sp. nov.

Branchlets closely subargenteous-tomentose; leaves linear, about 1.2 cm. long, glabrous above, densely and closely subargenteous-tomentose beneath, short-petioled, subacutely callous-tipped; heads solitary, subsessile, large; involucre 10 mm. high, the outer phyllaries densely whitish-tomentose, the inner linear, obtuse, slightly ampliate at apex, with deeply lacerate-ciliate scarious margin; achenes sparsely hirsute and glandular.

Low shrub, fastigiate branched, glabrescent in age, the petiole bases persistent; petioles 1 mm. long; blades more or less spreading, 8-15 mm. long, 1-1.5 mm. wide, shining above, sometimes tomentulose at base of costa, rather strongly revolute, the costa concealed beneath; heads solitary at tips of branchlets, about 2.5 cm. wide; disk 8-10 mm. high; involucre strongly graduate, about 6-seriate, the outer phyllaries triangular, acute, lacerate-ciliate toward apex, the inner linear, thinly pilose or glabrate, the

body 0.6–0.8 mm. wide, purplish-brown, its deeply lacerate-ciliate, brownish-white, scarious margin about as wide; receptacle alveolate; rays about 35, "pale hortense violet," the tube sparsely puberulous, 1.5 mm. long, the lamina linear, bidenticulate, 1.3 cm. long, 1.5 mm. wide; disk corollas numerous, purple above, sparsely pilosulous, 5.8 mm. long (tube 1 mm., throat cylindric-funnelform, 4 mm., teeth 0.8 mm. long); achenes sparsely hirsutulous and especially above glandular, 2 mm. long; pappus barely purplish-tinged, 4.8 mm. long, some of the inner bristles slightly thickened above, the outer pappus setulose-squamellate, 1.2 mm. long; stigmatic region 0.5 mm. long, the style appendages subulate, acuminate, 1.2 mm. long, short-hirsute.

PERU: Rocky banks on páramo, Paso de Tres Cruces, Cerro de Cusiluyoc, Dept. Cuzco, alt. 3800–3900 m., 3 May 1925, *Pennell* 13858 (type no. 558144, Field Mus.; photog. and fragm., U. S. Nat. Herb.).

Allied to *D. pearcei* and *D. hippophae*; distinguished from the latter by the lack of setulae on the branches, and from both by the glabrous upper surface of the leaves and the apically somewhat ampliate, elegantly lacerate-ciliate inner phyllaries.

8. *Diplostephium hippophae* Blake, sp. nov.

Branches closely whitish-tomentose and densely setulose with short, thick, spreading, yellowish hairs, these long-persistent; leaves linear, about 1.5 cm. long, acutely callous-pointed, short-petioled, above green, antrorse-spinulose along costa and often along margin, beneath densely silvery-tomentose with a very closely matted tomentum, narrowly revolute-margined; heads solitary, large; involucre 13 mm. high.

Shrub 1 m. high, fastigiate-branched above, the branches simple; internodes 1–3 mm. long; petioles 1 mm. long; leaves spreading, 1.2–1.8 cm. long, 1–2 mm. wide, the costa rather narrow, usually naked beneath; the upper leaves often bearing axillary fascicles of reduced leaves; head apparently sessile, about 2.5 cm. wide; disk 1.4 cm. high; involucre strongly graduate, 7–8-seriate, the outer phyllaries (about 4 rows) triangular-ovate, acute, closely whitish-tomentose, glabrescent, the inner linear, acute or subacuminate, lacerate-ciliate at apex, on back glabrate or sparsely pilosulous, all indurate and brownish-white; rays 20 or more, "purplish," hirsutulous on tube above, the tube 2–2.5 mm. long, the lamina linear, tridenticulate, 4-nerved, 14 mm. long, 1.5 mm. wide; disk corollas rather numerous, purple, hirsutulous chiefly at base of throat, 7.7 mm. long (tube 2 mm., throat 4.5 mm., teeth 1.2 mm.); achenes sparsely hirsutulous and toward apex sparsely glandular, 2–2.8 mm. long, those of the disk at least sometimes fertile; pappus purple-tinged, 6–7 mm. long, somewhat graduate, the longer bristles somewhat thickened at apex, the outer pappus sparse, setulose, about 0.5 mm. long; style branches linear or linear-subulate, hirsutulous above.

PERU: Grassy slope, Villcabamba, an hacienda on Río Chinchao, Dept. Huánuco, alt. 1830 m., 17–26 July 1923, *Macbride* 5136 (type no. 536184, Field Mus.; photog. and fragm., U. S. Nat. Herb.).

9. *DIPLOSTEPHIUM ANACTINOTUM* Wedd. Chlor. And. 1: 201. pl. 35, f. B. 1857; Blake, Contr. U. S. Nat. Herb. 24: 72. 1922.

RANGE: Sierra Nevada of Santa Marta, Colombia.

ADDITIONAL SPECIMEN EXAMINED: COLOMBIA: Páramo, San Miguel, Sierra Nevada de Santa Marta, alt. 3000 m., *Karsten* (herb. Sch. Bip., fragm. N).

Easily recognized by its small, elliptic or oval, thick leaves, 5–10 mm. long, 2–4 mm. wide (including the petiole, this 1–2.5 mm. long), covered with a viscid coat above, and its very short ligules surpassed by the styles. The labels of the specimens in Schultz's herbarium bear an unpublished generic name referring to the fragrant quality of the leaves.

10. *DIPLOSTEPHIUM PARVIFOLIUM* Blake, Contr. U. S. Nat. Herb. 24: 74. 1922.

Diplostephium microphyllum Wedd. Chlor. And. 1: 201. 1857. Not *D. microphyllum* Nees, 1832.

Linochilus microphyllus Sch. Bip.; Wedd. Chlor. And. 1: 201. 1857, as synonym.

RANGE: Sierra Nevada of Santa Marta, Colombia.

SPECIMENS EXAMINED: COLOMBIA: Santa Marta, alt. 3200 m., Jan. 1843, *Funck* 477 (herb. Sch. Bip., fragm. N); same locality, *Galeotti* 388 (herb. Sch. Bip., ex herb. Turcz.; presumably of the type collection).

The affinity of this species is with *D. anactinotum*, as is indicated by its short rays (the lamina 3 mm. long, according to Weddell), and the densely glandular upper surface of its closely imbricate, sessile leaves. The immature achenes are strongly compressed and rather sparsely appressed-pubescent, and the outer pappus is composed of slender setae 1–2 mm. long.

11. *DIPLOSTEPHIUM SPINULOSUM* Wedd. Chlor. And. 1: 200. 1857.

RANGE: Colombia, Ecuador.

SPECIMENS EXAMINED: COLOMBIA: Shrub zone (páramillo), Mt. Pan de Azucar, Cordillera Central, Dept. Cauca, alt. 3300–3600 m., 16 June 1922, *Pennell* 7041 (N). ECUADOR: Alpine pastures, Andes of Quito, alt. 4270 m., *Jameson* 406 (type, Par.; photog. and fragm. N).

The confusion of this species with *D. glandulosum* by Hieronymus and the writer has been discussed under species no. 3 of this revision. *Diplostephium spinulosum* is easily recognized by its blackish tomentum and its small, oblong or oblong-ovate leaves borne on closely appressed petioles. The spinules of the upper leaf surface are nearly absent in Pennell's plant. The rays in this collection are described as white, the disk as yellow. The achenes are sparsely glandular and hispidulous, and the outer pappus consists of setiform paleae 0.5–1 mm. long.

12. *DIPLOSTEPHIUM ADENACHAENIUM* Blake, Contr. U. S. Nat. Herb. 24: 72. 1922.

RANGE: Colombia, Ecuador.

ADDITIONAL SPECIMENS EXAMINED: COLOMBIA: Shrub zone (páramillo), Mt. Pan de Azucar, Cordillera Central, Dept. Cauca, alt. 3300–3600 m., 16

June 1922, *Pennell* 7035 (N). Mt. Azufra, Tuquerres, alt. 3800 m., *Karsten* (herb. Sch. Bip., fragm. N). ECUADOR: Mirador, July 1902, *Rivet* 182 (Par.). El Angel to La Posta (?), alt. 3500 m., Feb. 1903, *Rivet* 408 (Par.).

Readily recognized by its small, shining, elliptic to linear-oblong, distinctly petioled leaves, and densely glandular achenes. *Pennell* describes the rays as purple-violet; *Rivet* gives the flower color in one case as white, in the other as blue. *Karsten's* plant bears an unpublished specific name of *Schultz Bipontinus*.

13. *DIPLOSTEPHIUM MEYENII* Wedd. Chlor. And. 1: 201. 1857; Blake, Contr. U. S. Nat. Herb. 24: 72. 1922.

Linochilus meyenii Sch. Bip.; Wedd. Chlor. And. 1: 201. 1857, as synonym.

?*Aster* (?) *trachyticus* F. Phil.; Phil. Anal. Mus. Nac. Chile Bot. 1891: 37. 1891.

RANGE: Definitely known only from the type locality, Cordillera of Tacora, altitude 4000-4500 m., Department of Tacna, Peru [now Chile?].

This species and the following one are closely allied if not identical. Weddell's description is not sufficiently detailed to enable the two to be distinguished satisfactorily. The following specimens, all from the Department of Arequipa, Peru, alt. 2600-3700 m., belong to *D. tacorense* or *D. meyenii*: *Rose* 18954 (N), *Pennell* 13202 (F), 13213 (F), 13261 (F), 14272 (F). A closely related but possibly distinct plant, with oblanceolate leaves about 1.5 cm. long and 3 mm. wide, and involucre 10 mm. high, is represented by *Pennell* 13333 (F), from the same region.

14. *DIPLOSTEPHIUM TACORENSE* Hieron. Bot. Jahrb. Engler 21: 337. 1895; Blake, Contr. U. S. Nat. Herb. 24: 72. 1922.

RANGE: Known only from the type locality in northern Chile (Tacora).

SPECIMEN EXAMINED: CHILE: Tacora, alt. 4270-5185 m., April 1831, *Meyen* (type, fragm. N).

15. *DIPLOSTEPHIUM JELSKII* Hieron. Bot. Jahrb. Engler 36: 376. 1905; Blake, Contr. U. S. Nat. Herb. 24: 73. 1922.

RANGE: Known only from the type locality, Cutervo, Dept. Caxamarca, Peru.

SPECIMEN EXAMINED: PERU: Cutervo, May 1879, *de Jelski* 622 (type, fragm. N).

Distinguished by its small, rather acutely callous-pointed, linear or "linear-lanceolate" leaves, glabrous and shining above, and very strongly revolute-margined.

16. *Diplostephium gnidioides* Blake, sp. nov.

Branches loosely cinereous- or in age fuscous-tomentose and yellowish-setulose; leaves linear to narrowly lance-linear, about 8 mm. long, acutely

callous-pointed, short-petioled, spinulose above, usually so strongly revolute-margined as to conceal the loose grayish tomentum beneath; heads solitary, medium-sized, subsessile; involucre about 8 mm. high; achenes sparsely hirsutulous and toward apex sessile-glandular.

Shrub 30–60 cm. high, fastigiately branched, the petiole bases persistent, the branches glabrescent; internodes 1–3 mm. long; petioles about 1 mm. long; blades spreading or erectish, 6–11 mm. long, 0.6–2 mm. wide, above green, sparsely or usually densely spinulose, often with axillary fascicles; disk 8 mm. high; involucre strongly graduate, about 5-seriate, the phyllaries lance-ovate to linear, acute or subacuminate, indurate, whitish brown, rather densely and subpersistently cinereous-pilose-tomentose and ciliate; rays essentially glabrous, the tube 2 mm. long, the lamina linear, bidenticulate, 3–4-nerved, 13 mm. long, 1.3 mm. wide; disk corollas purple above, puberulous on throat and teeth, 6.3 mm. long (tube 2.5 mm., throat thick-cylindric, 3 mm., teeth 0.8 mm.); achenes 1.8 mm. long, those of disk 5-nerved; pappus brownish white or purple-tinged, 5 mm. long, the inner bristles slightly thickened apically, the outer pappus setulose, 0.5–1 mm. long; stigmatic region 0.5 mm. long, the linear-subulate, acuminate, hispidulous appendages 1.6 mm. long.

PERU: Grassy uplands, 9.6 km. south of Mito, Dept. Huánuco, alt. 3050 m., 1–5 Aug. 1922, *Macbride & Featherstone* 1858 (type no. 518353, Field Mus.; duplicate no. 1121760, U. S. Nat. Herb.). On open slope, Villcabamba, on Río Chinchao, Dept. Huánuco, alt. 1830 m., 17–26 July 1923, *Macbride* 5184 (F, N).

17. *DIPLOSTEPHIUM LAVANDULIFOLIUM* H. B. K. Nov. Gen. & Sp. 4: 97.

pl. 335. 1820; Wedd. Chlor. And. 1: 199. *pl.* 36, *f.* A. 1857.

Blake, Contr. U. S. Nat. Herb. 24: 74. 1922.

Diplopappus lavandulifolius Cass. Dict. Sci. Nat. 25: 96. 1822.

Aster lavandulaceus Willd.; Nees, Gen. & Sp. Ast. 189. 1832, as synonym.

Linochilus lavandulifolius Sch. Bip.; Wedd. Chlor. And. 1: 200. 1857, as synonym.

RANGE: Ecuador.

SPECIMEN EXAMINED: ECUADOR: [Near Mulalo, alt. 2930 m., near base of Mt. Illinissa and Mt. Cotopaxi,] *Bonpland* 3064 (type, herb. Humb. & Bonpl.).

18. *DIPLOSTEPHIUM HARTWEGII* Hieron. Bot. Jahrb. Engler 21: 337.

1895; Blake, Contr. U. S. Nat. Herb. 24: 74. 1922.

RANGE: Colombia, Ecuador.

ADDITIONAL SPECIMENS EXAMINED: ECUADOR: Saraguro Mountains, *Hartweg* 763 (type, fragm. N). Andine region, Mt. Pichincha, 4000 m., 1917, *Mille* (N).

Described by Father Mille as a shrub 2–3 meters high.

19. *DIPLOSTEPHIUM ANTISANENSE* Hieron. Bot. Jahrb. Engler 21: 338.

1895; Blake, Contr. U. S. Nat. Herb. 24: 74. 1922.

Diplostephium pycnophyllum Blake, Contr. U. S. Nat. Herb. 24: 75. 1922.

RANGE: Ecuador.

SPECIMENS EXAMINED: ECUADOR: Páramos of Cerro Antisana, near Las Cimarronas, alt. 4000 m., Oct. 1871, *Stuebel* 235a (type, fragm. N). Páramo near Cañar, 16 Sept. 1918, *Rose & Rose* 22750 (type of *D. pycnophyllum*, N). Páramo between Cuenca and Huigra, Sept. 1923, *Hitchcock* 21661 (N).

Distinguished from *D. lavandulifolium* and *D. hartwegii* by its smaller heads and shorter involucre. Hitchcock describes the plant as a shrub 0.3-1 meter high, with white rays.

SERIES 2. ROSMARINIFOLIA Blake, Contr. U. S. Nat. Herb. 24: 69. 1922.

Heads several to many at tips of stem and branches, cymose or cymose-panicled; leaves linear or slightly spatulate-linear, 2 mm. wide or less (rarely slightly wider in no. 21).

20. DIPLOSTEPHIUM REVOLUTUM Blake, Contr. U. S. Nat. Herb. 24: 78. 1922.

RANGE: Vicinity of Bogotá, Colombia.

ADDITIONAL SPECIMEN EXAMINED: COLOMBIA: Moist páramo near Laguna Verjon, near Bogotá, alt. 3200-3400 m., 27 Sept. 1917, *Pennell* 2263 (N).

Unique in its group in its essentially glabrous branchlets and pedicels. The rays are described as white. Pennell's plant was distributed as a *Baccharis*, which it much resembles in appearance.

21. DIPLOSTEPHIUM CARABAYENSE Wedd. Chlor. And. 1: 202. 1857; Blake, Contr. U. S. Nat. Herb. 24: 79. 1922.

RANGE: Known only from the type locality, in alpine region of southeastern Peru.

SPECIMEN EXAMINED: PERU: Near San Juan del Oro, Prov. Carabaya, June-July 1847, *Weddell* 4648 (type, Par.; photog. and fragm. N).

Distinguished in its group by its large heads and comparatively small, linear leaves (8-15 mm. long, 1.5-2 mm. wide), green and glabrous above, densely lanate-tomentose beneath with ochroleucous or whitish hairs. Weddell's label gives the color of the flowers as pale violet or lilac.

22. DIPLOSTEPHIUM CYPARISSIAS Wedd. Chlor. And. 1: 203. 1857; Blake, Contr. U. S. Nat. Herb. 24: 77. 1922.

RANGE: Sierra Nevada of Santa Marta, Colombia.

SPECIMEN EXAMINED: COLOMBIA: Sierra Nevada of Santa Marta, alt. 2745 m., *Funck* 387 (type, Par.; photog. and fragm., N).

This species, known only by the original collection, has leaves 1.5-2 cm. long, 1.5-2 mm. wide. The flowers are described as white.

23. DIPLOSTEPHIUM BACCHARIDEUM Blake, Contr. U. S. Nat. Herb. 24: 77. pl. 25. 1922.

RANGE: Colombia.

ADDITIONAL SPECIMENS EXAMINED: COLOMBIA: Without definite

locality, *Triana* 1263 (Brit. Mus.). Páramo de Choachi, near Bogotá, alt. 3700 m., 8 Aug. 1922, *Killip & Ariste-Joseph* 11927 (N). Shrub zone below páramo, Cerro Tatama, Cordillera Occidental, Dept. Caldas, alt. 3300–3500 m., Sept. 1922, *Pennell* 10535 (N).

The flowers are described as white (no. 11927). Pennell gives the vernacular name "romero" for this shrub.

24. *DIPLOSTEPHIUM ROSMARINIFOLIUM* (Benth.) Wedd. Chlor. And. 1: 202. 1857; Blake, Contr. U. S. Nat. Herb. 24: 77. 1922.

Linochilus rosmarinifolius Benth. Pl. Hartw. 197. 1845.

RANGE: Known only from the type collection from near Bogotá, Colombia.

Closely similar to *D. baccharideum*, but with considerably long and somewhat broader leaves.

- SERIES 3. *RUPESTRIA* Blake, Contr. U. S. Nat. Herb. 24: 69. 1922.

Heads several to many at tips of stem and branches, cymose or cymose-panicked; leaves linear-lanceolate to oblong or obovate, rarely linear, 3–30 mm. wide.—Including Series *Floribunda* and *Denticulata* Blake, Contr. U. S. Nat. Herb. 24: 69. 1922.

25. *DIPLOSTEPHIUM WEDDELLII* Blake, Contr. U. S. Nat. Herb. 24: 79. 1922.

Diplostephium sessiliflorum Wedd. Chlor. And. 1: 204. 1857. Not *D. sessiliflorum* Spreng. 1826.

RANGE: Sierra Nevada of Santa Marta, Colombia.

SPECIMENS EXAMINED: COLOMBIA: Sierra Nevada, Prov. Río Hacha, alt. 3660–4120 m., March 1852, *Schlim* 806 (type, Par.; photog. and fragm. N). San Miguel, Sierra Nevada of Santa Marta, *Karsten* (herb. Sch. Bip., fragm. N).

In Karsten's plant the heads are apparently solitary in some cases.

26. *DIPLOSTEPHIUM RUPESTRE* (H. B. K.) Wedd. Chlor. And. 1: 206. 1857; Blake, Contr. U. S. Nat. Herb. 24: 79. 1922.

Aster rupestris H. B. K. Nov. Gen. & Sp. 4: 94. pl. 334. 1820.

Tetramolopium ? rupestre Nees, Gen. & Sp. Ast. 203. 1832.

Aster pichinchensis Willd.; Nees, Gen. & Sp. Ast. 203. 1832, as synonym.

RANGE: Colombia, Ecuador.

ADDITIONAL SPECIMENS EXAMINED: COLOMBIA: Shrub-forest on slope, Páramo del Quindio, Dept. Caldas, alt. 4200–4400 m., Aug. 1922, *Pennell & Hazen* 9907 (N); grassy páramo, alt. 4100–4400 m., same locality, *Pennell & Hazen* 9835 (N). ECUADOR: Rucú-Pichincha, *Bonpland* (type, Par.). Mt. Pichincha, alt. 4000 m., *André* 3911 (Field Mus.). Mt. Pichincha, alt. 4100–4500 m., Aug. 17, 1923, *Hitchcock* 21087 (N). Mt. Rumiñahui, alt. 4300 m., Aug. 1917, *Mille* (N).

The rays in this species are white, the disk corollas "yellow brown" or "purple," according to Pennell & Hazen's labels.

27. *DIPLOSTEPHIUM ERIOPHORUM* Wedd. Chlor. And. 1: 206. *pl.* 36, *f.* C. 1857; Blake, Contr. U. S. Nat. Herb. 24: 79. 1922.

RANGE: Departments of Tolima and Caldas, Colombia.

ADDITIONAL SPECIMEN EXAMINED: COLOMBIA: Shrub forest on slope, Páramo del Quindio, Cordillera Central, Dept. Caldas, alt. 4200-4400 m., Aug. 1922, *Pennell & Hazen* 9908 (N).

The label describes the rays as white and the disk as purple.

28. *DIPLOSTEPHIUM LECHLERI* (Sch. Bip.) Wedd. Chlor. And. 1: 204. 1857; Blake, Contr. U. S. Nat. Herb. 24: 81. 1922.

Liabum (Oligactis) lechleri Sch. Bip. Bonplandia 3: 236. 1855.

RANGE: Known only from the type collection from near Sachapata, Andes of Carabaya, Peru.

ADDITIONAL SPECIMEN EXAMINED: PERU: Sachapata, Prov. Carabaya, Aug. 1854, *Lechler* 2517 (type coll.; Par., photog. and fragm. N).

29. *Diplostephium pulchrum* Blake, sp. nov.

Branches thinly and closely silvery-tomentose and densely hirsute-pilose with many-celled, spreading, yellowish-white hairs; leaves lance-linear, about 5 cm. long, finely and evenly hispidulous above, thinly and closely argenteous-tomentose beneath; heads large, in a terminal leafy-bracted cyme; involucre about 14 mm. high; pappus purple.

"Small tree, 3.6 m. high," or "shrub, 1.3-2 m. high," the branchlets fastigate, at length glabrate or glabrescent, 2-3 mm. thick; internodes 1-5 mm. long; petioles broad, 1-2 mm. long; blades lance-linear or elliptic-linear, 4-6.5 cm. long, 5-8 mm. wide, acuminate callous-tipped, truncate-rounded at base, usually only slightly revolute, entire, thin-coriaceous, above deep green, shining, impressed-veined, the costa prominent and nearly glabrous beneath, the lateral veins 15-25 pairs, delicate, spreading nearly at a right angle, forked and anastomosing; heads about 2.5 cm. wide, in nearly or quite simple cymes of 9-14 toward apex of branches, the peduncles 1-7 cm. long, pubescent like the branches, bracted with linear strongly revolute-margined leaves about 2 cm. long; disk in fruit 1-1.2 cm. high; involucre graduate, about 6-seriate, the phyllaries linear-subulate, 0.8-1 mm. wide, attenuate, thinly tomentose, scarcely ciliate, with loose subcirrhous yellowish tips; receptacle alveolate, the alveolae lacerate-fringed; rays "white," about 54, the tube puberulous toward apex, 2 mm. long, the lamina linear, bidenticulate, 4-nerved, 15 mm. long, 1.5 mm. wide; disk corollas very numerous, purple above, puberulous, 6.5 mm. long (tube 2 mm., throat cylindric, 3.5 mm., teeth 1 mm.); achenes sparsely hirsutulous and glandular, 5-nerved, 2 mm. long; pappus deep purplish except at base, the inner of bristles 5 mm. long, scarcely thickened apically, the outer setulose-squamellate, whitish, 1 mm. long; style branches with subulate, acuminate, dorsally hispidulous appendages nearly or quite as long as the stigmatic region.

PERU: Wet mossy rocky open uplands, Tambo de Vaca, Dept. Huánuco, alt. 3965 m., 10-24 June 1923, *Macbride* 4346 (type no. 535431, Field Mus.;

duplicate no. 1,191,499, U. S. Nat. Herb.). Muña, Dept. Huánuco, alt. 2745-3050 m., May 1863, R. Pearce (K, fragm. N).

In addition to the characters mentioned in the key, the species differs from *D. lechleri* in its involucre. In that plant the involucre is only 9-10 mm. high, and there are 3 or 4 outer series of triangular-ovate phyllaries 4 mm. long or less, while in *D. pulchrum* even the outermost phyllaries are linear-subulate and 6-8 mm. long.

30. *DIPLOSTEPHIUM HAENKEI* (DC.) Wedd. Chlor. And. 1: 203. 1857; Blake, Contr. U. S. Nat. Herb. 24: 83. 1922.

Simblocline haenkei DC. Prodr. 5: 297. 1836.

Diplostephium affine Wedd. Chlor. And. 1: 203. 1857; Blake, Contr. U. S. Nat. Herb. 24: 86. 1922.

Diplostephium mandonii Sch. Bip. Linnaea 34: 534. 1865-66, and Bull. Soc. Bot. France 12: 81. 1865, nomen nudum.

Aster sejaense Kuntze, Rev. Gen. Pl. 3²: 131. 1898.

Diplostephium mandoni Rusby, Bull. N. Y. Bot. Gard. 4: 383. 1907.

Diplostephium liabioides Rusby, Bull. N. Y. Bot. Gard. 4: 384. 1907.

Diplostephium atropurpureum Rusby, Bull. N. Y. Bot. Gard. 4: 384. 1907.

Diplostephium sejaense Blake, Contr. U. S. Nat. Herb. 24: 80. 1922.

RANGE: Peru, Bolivia.

ADDITIONAL SPECIMENS EXAMINED: PERU: Without definite locality, *Haenke* (type of *S. haenkei*, Prodr. Herb.). Cordillera of Carabaya, alt. 3000-3500 m., 1847, *Weddell* 4740 (type of *D. affine*, Par.; photog. and fragm. N). Rocky thicket, shrub zone, alt. 3500-3800 m., Paso de Tres Cruces, Cerro de Cusilluyoc, Dept. Cuzco, 1925, *Pennell* 13899 (F). BOLIVIA: Near Sorata, *Mandon* 215 (chirotype of *D. mandonii* Sch. Bip., herb. Sch. Bip.; fragm. N.).

Comparison of the types of *D. haenkei* and *D. affine* with material of *D. sejaense* by the writer in 1925 disclosed no differences of any moment. Buchtien's labels (nos. 3030, 3031) describe the plant as a shrub 2 meters high, with violet flowers. The heads are rarely solitary.

31. *DIPLOSTEPHIUM PHYLICOIDES* (H. B. K.) Wedd. Chlor. And. 1: 205. 1857; Blake, Contr. U. S. Nat. Herb. 24: 80. 1922.

Aster phyllicoides H. B. K. Nov. Gen. & Sp. 4: 93. 1920.

Tetramolopium ? *phyllicoides* DC. Prodr. 5: 262. 1836.

Linochilus phyllicoides Sch. Bip.; Wedd. Chlor. And. 1: 205. 1857, as synonym.

Diplostephium umbelliferum Blake, Contr. U. S. Nat. Herb. 24: 80. pl. 26. 1922.

RANGE: Vicinity of Bogotá, Colombia.

ADDITIONAL SPECIMENS EXAMINED: COLOMBIA: Páramo de Choachi, near Bogotá, alt. 3700 m., 8 Aug. 1922, *Killip & Ariste-Joseph* 11926 (N).

Bogotá, *Karsten* (herb. Sch. Bip., fragm. N). Without definite locality ("Nova Hispania"), *Bonpland* (type of *A. phyllicoides*, herb. Humb. & Bonpl.).

This species is distinguished by its small, thick, elliptic or oblong leaves and umbelliform clusters of small heads. The flowers are described as white in no. 11926; those of other collections have been described as purple blue. The characters supposed to distinguish *D. umbelliferum* from *D. phyllicoides* are of no consequence. The style branches tend to adhere, and the supposed difference in shape was evidently due principally to the degree to which they were separated in dissecting.

32. *DIPLOSTEPHIUM SCHULTZII* Wedd. Chlor. And. 1: 204. 1857; Blake, Contr. U. S. Nat. Herb. 24: 83. 1922.

Linochilus iodopappus (*jodopappus*) Sch. Bip.; Wedd. Chlor. And. 1: 204. 1857, as synonym.

RANGE: Colombia.

ADDITIONAL SPECIMENS EXAMINED: COLOMBIA: Volcán de Tolima, alt. 4025 m., Jan. 1843, *Linden* 901 (type collection; Par., herb. Sch. Bip., photog. and fragm. N). Along stream, Páramo del Quindio, Cordillera Central, Dept. Caldas, alt. 4100-4400 m., Aug. 1922, *Pennell & Hazen* 9825 (G, N.). Shrub-zone, below páramo, Cerro Tatama, Cordillera Occidental, Dept. Caldas, alt. 3300-3500 m., Sept. 1922, *Pennell* 10532 (G, N). Without definite locality, *Triana* 90 (G).

This species and the next, which are very closely allied, are most closely related to the group consisting of *D. lehmannianum*, *D. rhododendroides*, and *D. pleistogynum*, and are distinguished principally by the persistent tomentum of the upper leaf surface. *Linden's* plant is described as purple-flowered. In one of *Pennell's* plants the disk is described as red, and the rays pink; in the other the rays are said to be blackish-violet, although they appear to have been of the same color as in the first specimen. The pappus and disk corollas become deep purple at maturity. In a head of no. 9825, many of the alveolar margins of the receptacle were prolonged into slender smooth setae up to 1 mm. long. Reëxamination of *Triana* 90 shows that it belongs to *D. schultzii* and not to *D. cochense*, to which I previously referred it.

A head of the type collection dissected by the writer contained about 44 rays and 6 disk flowers. The two collections by *Pennell*, which are certainly specifically identical with each other and with the type, differ remarkably in the composition of the heads. In one (no. 9825) two heads dissected, from different specimens, contained in one case 37 rays and 4 disk flowers, and in the other 10 rays and 32 disk flowers. Four heads of no. 10532, very carefully dissected from sheets in two different herbaria, showed respectively 38, 32, 31, and 27 rays, and no disk flowers whatever! The heads appeared normal, the rays were normally formed, although often shorter than the

styles, and none showed any sign of stamens; the mature achenes were normal in appearance, but their embryos were abortive. This collection apparently represents a remarkable mutation, of a sort I have never before met with in Compositae. Its significance is discussed in the introduction to this paper.

33. *DIPLOSTEPHIUM COCHENSE* Hieron. Bot. Jahrb. Engler 21: 341. 1895; Blake, Contr. U. S. Nat. Herb. 24: 83. 1922.

RANGE: Colombia.

SPECIMENS EXAMINED: COLOMBIA: Llañura del Río Cocha, Aug. 1869, *Stuebel* 353 (type, fragm. N.).

Triana 90, doubtfully referred to this species in my former paper, proves on reexamination to belong to *D. schultzii*.

34. *DIPLOSTEPHIUM COSTARICENSE* Blake, Contr. U. S. Nat. Herb. 24: 82. pl. 27. 1922.

RANGE: Mountains and páramos of Costa Rica, altitude 1500-3100 meters.

ADDITIONAL SPECIMENS EXAMINED: COSTA RICA: Common, wet páramo thicket, Cerro de las Vueltas, Prov. San José. alt. 2700-3000 m., Dec. 1925-Jan. 1926, *Standley & Valerio* 43722, 43809 (N).

Described as a dense gray shrub 3-4.5 m. high, with white rays and dark purple-red disk. The heads in this species are 22-57-flowered, with 16-27 rays and 6-36 disk flowers. In no. 43722 the ray corollas are normally formed but very short (the lamina 1.7-2 mm. long), equaling or shorter than the styles.

35. *DIPLOSTEPHIUM OBTUSUM* Blake, Contr. U. S. Nat. Herb. 24: 84. 1922.

RANGE: Páramos of the State of Trujillo, Venezuela, alt. 2800-3200 meters.

36. *DIPLOSTEPHIUM LEHMANNIANUM* Hieron. Bot. Jahrb. Engler 21: 340. 1895; Blake, Contr. U. S. Nat. Herb. 24: 82. 1922.

Diplostephium schultzii var. *lehmanniana* Hieron. Bot. Jahrb. Engler 19: 48. 1894.

RANGE: Colombia, Ecuador.

SPECIMENS EXAMINED: COLOMBIA: Highest shrub-zone, Páramo de Guanacas, Prov. Popayán, alt. 3000-3500 m., Jan., *Lehmann* 4893 (type, fragm. N). ECUADOR: Eastern andine region, Pifo, near Paluguillo, Prov. Pichincha, alt. 3800 m., 1898, *Mille* (N).

Distinguished, among the species with small leaves glabrous above, by its small heads and involucre. *Mille's* plant is described as a very handsome shrub 1.5 meters high.

37. *DIPLOSTEPHIUM RHODODENDROIDES* Hieron. Bot. Jahrb. Engler 21: 340. 1895; Blake, Contr. U. S. Nat. Herb. 24: 81. 1922.

RANGE: Known only from the type collection, from Azufral de Tuquerres, Colombia.

SPECIMEN EXAMINED: COLOMBIA: Laguna Verde, near Azufral de Tuquerres, Jan. 1870, *Stuebel* 429 (type, fragm. N).

38. *DIPLOSTEPHIUM PLEISTOGYNUM* Blake, Contr. U. S. Nat. Herb. 24: 82. 1922.

RANGE: Known only from the type collection, from Páramo de Buena Vista, Huila group, Cordillera Central, Dept. Cauca, Colombia.

39. *DIPLOSTEPHIUM FLORIBUNDUM* (Benth.) Wedd. Chlor. And. 1: 205. *pl.* 36, f. B. 1857.

Linochilus floribundus Benth. Pl. Hartw. 203. 1845.

Linochilus ochraceus Sch. Bip.; Wedd. Chlor. And. 1: 205. 1857, as synonym.

Diplostephium ochroleucum Klatt, Bot. Jahrb. Engler 8: 37. 1886.

Aster ochroleucus Kuntze, Rev. Gen. Pl. 3²: 131. 1898.

RANGE: Departments of Popayán and Cauca, Colombia.

ADDITIONAL SPECIMENS EXAMINED: COLOMBIA: Moist thicket in páramo ("llano"), Paletará, Cordillera Central, Dept. Cauca, alt. 2950-3100 m., June 1922, *Pennell* 6969 (N). Shrub-zone ("paramillo"), Mt. El Derrumbo, Cordillera Occidental, Dept. Cauca, alt. 2700-3000 m., June 1922, *Pennell* 7489 (N).

40. *Diplostephium leiocladum* Blake, sp. nov.

Branches, peduncles, and pedicels quickly glabrate; leaves elliptic or lance-elliptic, about 6 cm. long, repand-toothed or entire, glabrous above, whitish-tomentose beneath, slender-petioled; heads rather small, numerous, cymose-panicled; involucre about 7 mm. high.

Branches stout, 3-5 mm. thick, ridged by lines decurrent from the leaf bases, at first thinly but closely whitish-tomentose, soon nearly or quite glabrate, very leafy; internodes 2-4 mm. long; petioles slender, glabrous or quickly glabrate, very narrowly margined essentially to base, 8-10 mm. long; blades 4.5-7.5 cm. long, 1.2-1.8 cm. wide, acute, at base acuminate, repand-serrate (teeth acutish, about 3 mm. apart, 0.5-1 mm. high) or entire, coriaceous, narrowly revolute-margined, above deep green, shining, glabrous, beneath densely and closely whitish- or slightly ochroleucous-tomentose, the costa glabrous and prominent beneath, impressed above, the principal lateral veins 7-12 pairs, spreading at nearly a right angle, slender, prominulous, forked and anastomosing, usually becoming glabrate beneath, impressed above; heads about 13-15-flowered, in small pedunculate cymes in the axils of the uppermost leaves, forming a dense rounded panicle about 7 cm. wide, about equalled by the leaves, the peduncles pubescent like the stem and similarly glabrate, about 1.5 cm. long, the pedicels 3-7 mm. long; involucre narrowly campanulate, strongly graduate, about 5-seriate, the phyllaries ovate (outer) to linear-oblong or lance-linear, acute (outer) to obtuse,

indurate, obscurely greenish toward apex, scarious-margined, ciliolate and toward apex persistently tomentose; receptacle shallowly alveolate; rays about 5-8, "white," the tube sparsely puberulous, 4.5 mm. long, the lamina spreading, elliptic-linear, bidenticulate, 3-3.5 mm. long, 0.6 mm. wide; disk corollas about 8-10, whitish, densely puberulous on slender part of throat, the tube and greater part of throat isodiametric, 4 mm. long, the upper part of throat abruptly dilated for 0.5 mm., the lance-ovate teeth 1.5 mm. long; achenes glabrous or sparsely sessile-glandular above, 5-nerved, 1.8-2.5 mm. long, those of disk apparently infertile; pappus brownish white, 5 mm. long, the longer bristles slightly dilated at apex, the outer pappus sparse, setulose, 1 mm. long or less; style branches linear-oblong, hispidulous throughout, with short acute or subacuminate appendages.

COLOMBIA: Shrub zone, below páramo, Cerro Tatama, Cordillera Occidental, Dept. Caldas, alt. 3300-3500 m., 8-10 Sept. 1922, *Pennell* 10531 (type no. 1, 141, 316, U. S. Nat. Herb.); also *Pennell* 10530, on edge of páramo, same data.

Closely related to *D. floribundum*, but distinguished by its quickly glabrate peduncles and branches, as well as by its larger leaves. The leaves in no. 10531 are conspicuously toothed, in no. 10530 entire, but the two collections are clearly forms of one species.

41. *DIPLOSTEPHIUM DENTICULATUM* Blake, Contr. Gray Herb. 53: 25. 1918; Contr. U. S. Nat. Herb. 24: 85. 1922.

RANGE: Known only from the type locality, Guadalupe, Colombia, altitude 3000 meters.

Distinguished from the two following species by the conspicuous reticulation of the lower leaf surface and the pubescent achenes.

42. *DIPLOSTEPHIUM OCHRACEUM* (H. B. K.) Nees, Gen. & Sp. Ast. 201. 1832; Blake, Contr. U. S. Nat. Herb. 24: 85. 1922.

Aster ochraceus H. B. K. Nov. Gen. & Sp. 4: 85. 1820.

Tetramolopium ? ochraceum DC. Prodr. 5: 262. 1836.

RANGE: Known only from the type, collected "in montibus Quitensibus?"

SPECIMEN EXAMINED: ECUADOR (?): Without definite locality, *Bonpland* (type, herb. Humb. & Bonpl.; photog. N).

43. *DIPLOSTEPHIUM BICOLOR* Blake, Contr. U. S. Nat. Herb. 24: 85. pl. 28. 1922.

RANGE: Known only from the type locality, headwaters of the Río Lopez, Río Palo Basin, Tierra Adentro, Cauca, Colombia, altitude 2500-3000 meters.

Closely allied to *D. ochraceum*, and apparently distinguished principally by its more or less obovate, rather thicker leaves, with denser tomentum.

EXCLUDED SPECIES³

DIPLOSTEPHIUM CORYMBOSUM Donn. Smith, Bot. Gaz. 23: 8. 1897;
Blake, Contr. U. S. Nat. Herb. 24: 86. 1922.

This is ARCHIBACCHARIS CORYMBOSA (Donn. Smith) Blake, Journ.
Washington Acad. Sci. 17: 60. 1927 (*Hemibaccharis corymbosa* Blake,
Contr. U. S. Nat. Herb. 20: 553. 1924).

DIPLOSTEPHIUM INCANUM Hieron. Bot. Jahrb. Engler 21: 340. 1895;
Blake, Contr. U. S. Nat. Herb. 24: 85. 1922.

This is a species of the tribe Senecioneae, belonging to *Senecio* or a
related genus. The fragments of the type examined are insufficient to
enable the genus to be determined.

DIPLOSTEPHIUM PANICULATUM Donn. Smith, Bot. Gaz. 23: 8. 1897;
Blake, Contr. U. S. Nat. Herb. 24: 86. 1922.

This is *Archibaccharis mucronata* var. *paniculata* (Donn. Smith) Blake
(*Hemibaccharis mucronata paniculata* Blake, Contr. U. S. Nat. Herb. 20: 551.
1924; *Archibaccharis mucronata paniculata* Blake in Standl. Contr. U. S.
Nat. Herb. 23: 1509. 1926).

³ Including only species not disposed of in my 1922 revision.

GROWTH AND GERMINATION OF SUNFLOWERS AS INFLUENCED BY X-RAYS

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The manner in which the germination and growth of seeds are influenced by X-rays is a subject about which there is disagreement. The question to be determined is whether the action of X-rays on seeds and seedlings causes growth to be stimulated or inhibited. Is the effect on plants merely a transitory one or do the rays also exert an influence on the total amount of growth? In the experiments to be described a considerable number of plants were grown to maturity, so that the final, as well as the immediate, effects of irradiation could be determined.

A few of those who have studied the effects of X-rays on higher plants (1, 7, 10) report a positive stimulation by weak irradiation and find an acceleration of growth. One investigator (12) finds that X-rays exert a forcing action on certain buds while others (3, 6, 7) find a temporary checking of growth followed by an acceleration.

The facts reported in this paper suggest that plants from irradiated seeds show no increase in total growth either at an early or a later stage. The effect of the same dosage on air-dry and soaked seeds is discussed, as is also the variability of seeds of the same species in regard to their susceptibility to X-rays.

MOISTURE INTAKE OF SEEDS

The seeds of the so-called Russian sunflower, *Helianthus annuus*, were used for these experiments. The ovary wall was removed in all cases before irradiation. Air-dried seeds were found to contain 3.41 percent of water. Since some investigators (1, 3, 6, 7, 8, 10) have found that sensitivity to X-rays is influenced by the water content of the seeds, it was thought advisable to determine the water content of each lot of experimental seeds.

Seeds were placed in distilled water and immersed in a Freas water bath set at a temperature of 20° C. At definite periods they were removed from the water, dried quickly on filter paper, and weighed. They were then returned to the water bath until time for the next weighing. Fifty seeds were used in each determination. Table 1 gives the average of percentages of the original dry weight of the seeds determined from four sets of seeds.

In the experiments to be described, the seeds were always immersed in distilled water and placed in the water bath which was kept between 20° and 21° C. The length of time of soaking was noted and by use of table 1 the approximate amount of water present was determined. It may be noted

that after about 46 percent of water had been taken in, there was a marked depression in amount imbibed due to approaching saturation.

TABLE 1. *Water Intake of Seeds Kept at 20°-21° C. for Various Time Periods, Expressed as Averages of Percentages of the Original Dry Weight of Four Sets of Seeds*

Time Period in Hours	Water Absorbed, Percent	Time Period in Hours	Water Absorbed, Percent
1	24.65	12	56.52
1.75	32.75	13	58.17
3	40.22	14	59.22
4	46.76	16	59.52
5	48.46	17	59.99
6	49.57	22	60.65
7	52.00	28	60.78
8	52.34	34	62.68
9	52.64	46	64.21
10	53.80	51.3	66.31
11	54.81		

AMOUNT OF DOSAGE AFFECTING GROWTH OR GERMINATION

Determination of the dosage which, given to the seed, would in any way affect the growth of the seedling, was first necessary, for it is well known that different species of plants differ in their sensitivity to X-rays as well as to other short rays of light.

Effect of Certain Dosages upon Soaked Seeds as Determined by Growth Made During Early Stages

Stimulation of growth by means of weak doses of irradiation has been reported by investigators who based their conclusions on data taken during early stages of growth. In order to determine whether irradiation resulted in acceleration of growth in the early stages of growth of the sunflower seedling, measurements of height were taken. Average height of controls in all cases was a little greater than that of plants receiving weak irradiation.

For irradiation,¹ soaked seeds were placed in open petri dishes. They were so placed that the rays came directly from above. The unit of dosage used in these experiments is the "human erythema" dose of Ivy (4) and is designated by *E*. The "set-up" for one erythema dose is 60 K.V.M. (maximum kilovoltage), 5 milliamperes current, no filter, 30 cm. focal distance, 5 cm. portal of entry for 5½ minutes.

In the case of plants derived from seeds irradiated with weak dosage (1-5 *E*) little difference in the growth of young seedlings could be detected. When heavier dosage (10 *E*) was given, however, very decided inhibition of growth was evident in seedlings 14 days old. Table 2 records results from irradiated soaked seeds with the water content as stated. Measurements were taken from the base of the stem to the growing tip.

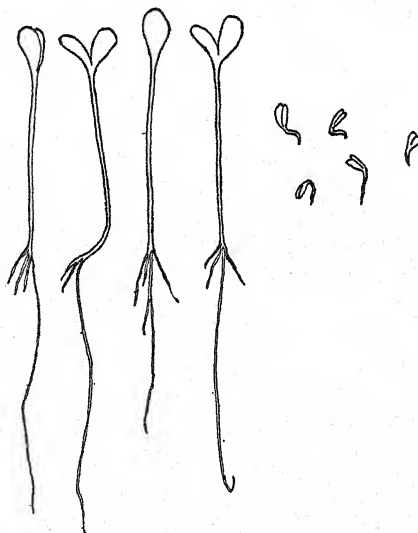
¹ The Standard X-ray Company of Chicago lent the equipment used in these experiments to Doctor A. C. Ivy, formerly of the Department of Physiology of the University of Chicago. Dr. Ivy kindly allowed the writer to use this equipment.

TABLE 2. *Effect of Dosage of X-rays upon Soaked Seeds as Determined by Data Taken During Early Stages of Growth. Results are Averages from 16 Plants*

Dose <i>E</i>	Percentage of Water Content of Seeds	Age of Plants at End of Experiment (Days)	Average Height (cm.)	
			Irradiated	Control
1	56.8	32	17.5	19
2	59.5	20	15.7	16
4	59.7	16	11.5	13
5	52.6	16	13.5	14
10	56.52	14	3.6	13.6

Effect of Certain Dosages upon Soaked Seeds as Determined by Percentage of Germination and by Data Taken at the End of the Life Cycle

Soaked seeds receiving light doses showed a slightly lower percentage of germination than controls. The total growth of the plants as measured by



TEXT FIG. 1. Six-day-old seedlings which were grown in sphagnum. At left, controls; at right, seedlings whose seeds were irradiated with 40 *E*. When seeds are subjected to a heavy dose (20–40 *E*) they never develop to a greater size than is shown here. (Drawn from a photographic reproduction.)

height and dry weight was depressed by irradiation. Seeds receiving a heavy dose (30 *E* or more) died after the cotyledons came through the soil (text fig. 1). Table 3 gives results of this series of irradiations. Data on percentage of germination were based on 20 plants in each lot. Lack of space in the greenhouse made it impossible to allow all of this number to complete their life cycles. Three or four plants for each group were carried to maturity. The height recorded includes measurement from base of stem to base of flower-head. The date of blooming recorded is the date when all the ray flowers had unfolded.

TABLE 3. *Effect of Various Doses on Soaked Seeds as Determined by Percentage of Germination, Date of Blossoming, and Total Growth at Maturity*

Series No.	Water Content of Seed	Dose	Percentage of Germination	Data Taken at Maturity		
				Height in cm.	Days to Blossoming	Air-dry Weight in gm.
AS. 2.....	56.8	1 E	83.3	100	118	13.15
Control.....	56.8	—	100	141	139	14.6
BS. 2.....	59.5	2 E	90	134	108	16.35
Control.....	59.5	—	90	158	121	18.28
CS. 2.....	59.7	4 E	60	116	112	7.805 †
Control.....	59.7	—	90	151	121	12.86
DS. 2.....	52.6	5 E	85	144	114	17.66
Control.....	52.6	—	90	153	127	18.20
HS. 2.....	62.04	30 E	95	*	*	
Control.....	62.04	—	100	110	90	

* Seedlings died after appearance of cotyledons above the soil.

† Absolute dry weight.

Results from the above and from various preliminary experiments show that, in general, plants from soaked irradiated seeds do not reach as great height as their controls but the time of blossoming comes at an earlier date. Weak doses of X-rays used on soaked sunflower seeds do not induce an acceleration which results in total increased growth at maturity.

EFFECTS ON SEEDLINGS PROPORTIONAL TO DOSAGE GIVEN SEEDS

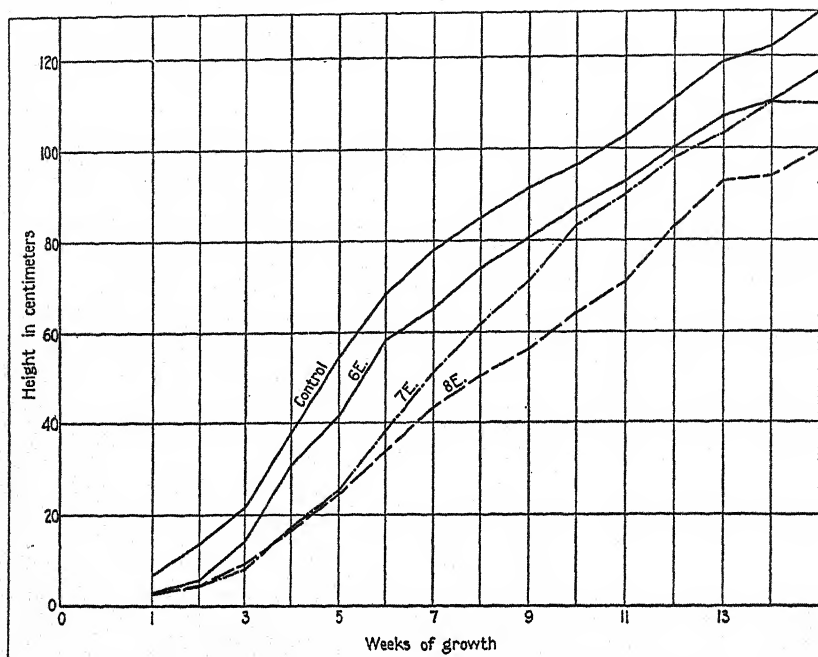
The amount of depression in growth during the first three weeks of seedlings derived from irradiated seeds is approximately proportional to the dosage. Later, if the death of the plant has not taken place, the growth-checking effects of irradiation tend to disappear. Those employing X-rays for therapeutic purposes find that a similar condition holds true for treatment of skin diseases. The dosage is repeated between the second and third weeks, for the effects of the treatment disappear by the end of the third week.

Seeds with a water content of 58.7 percent were exposed to a dosage of 5, 6, 7, 8, 9, and 10 E, respectively. In order to determine the relative effects of these doses, weekly measurements of the seedlings were taken. These, together with the number of days' growth before blossoming and absolute dry weights, are recorded in table 4. Results given are averages from four plants except from those receiving doses of 9 E and 10 E, respectively. In the case of the former, one seedling died at the end of four weeks and two seedlings derived from seeds which had received 10 E died during the same week. The seedlings from these two groups which were able to continue development after the fourth week showed themselves to be especially

TABLE 4. *Height of Plants from Irradiated Seeds, Taken at Weekly Intervals*

Series No.	Dose	Percentage Germi- nation	Percentage Living to Maturity	Height in cm. at Weekly Periods															Total Days' Growth Before Blossoming	Abs. Dry Wt. (gm.)
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
NS. 25...	5 E	95	100	5.3	9.4	18.5	34	44.7	57.4	62	68.5	74.8	80	86	91	98	98	105	7.078	
NS. 26...	6 E	90	100	2.9	5.6	14.1	31	41.5	58	65	74	80.6	87	93	100.4	107	110	117	103	7.493
NS. 27...	7 E	85	100	2.8	4.3	8	17	25.0	38	51	61.5	71.0	83	90	97.9	103	110	110	93	7.811
NS. 28...	8 E	100	100	2.5	4.4	9	16.7	24.5	34	43	50.0	56	64	71	83.0	93	94	100	99	6.468
NS. 29...	9 E	85	75	2.0	3.2	7.6	15	30	43	54	61	68.5	75	83	93.0	103	107	107	95	13.92
NS. 210...	10 E	95	50	1.9	2.3	4.7	13	26	42	52	60	68.0	75	84	95.7	111	123	129	108	21.46
Control....	—	100	100	6.5	13.4	21.5	38	54.5	68.4	78	85	91.5	96.5	10.3	111.1	119	122	130	101	8.918

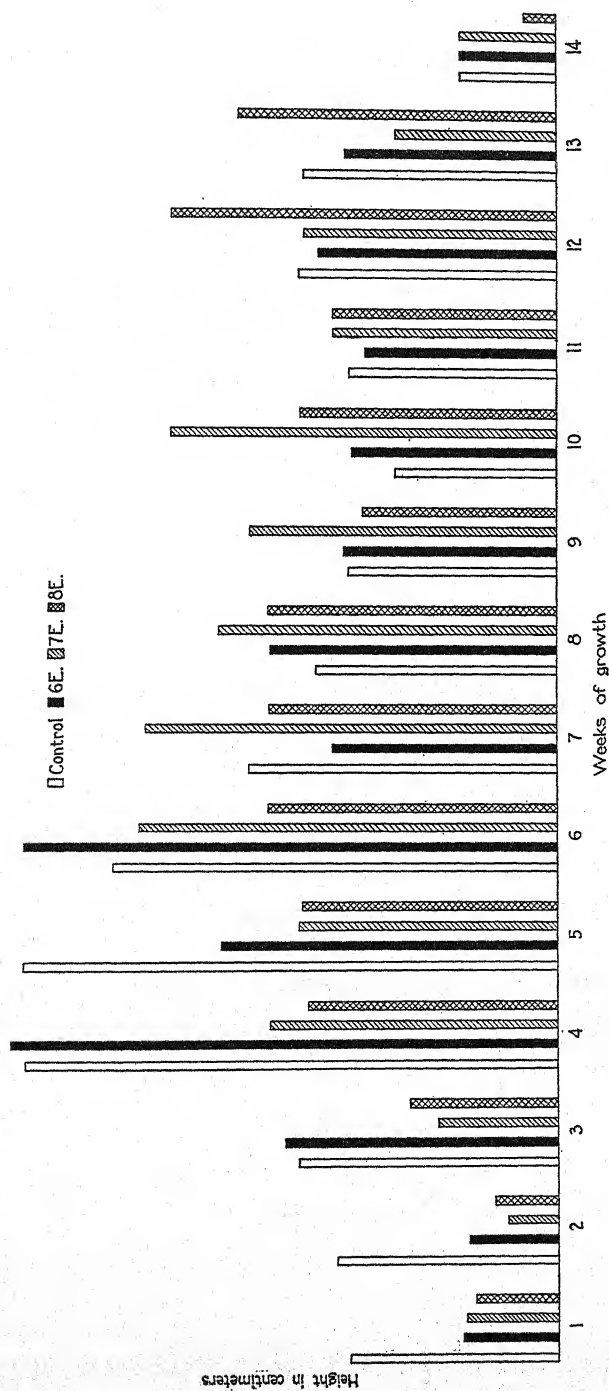
strong and thrifty. Their average absolute dry weight much exceeded the average weight of the control plants.



TEXT FIG. 2. Curves showing total growth of control plants and those from seeds which were irradiated with doses of 6, 7, and 8 *E*, respectively; growth measured at weekly intervals.

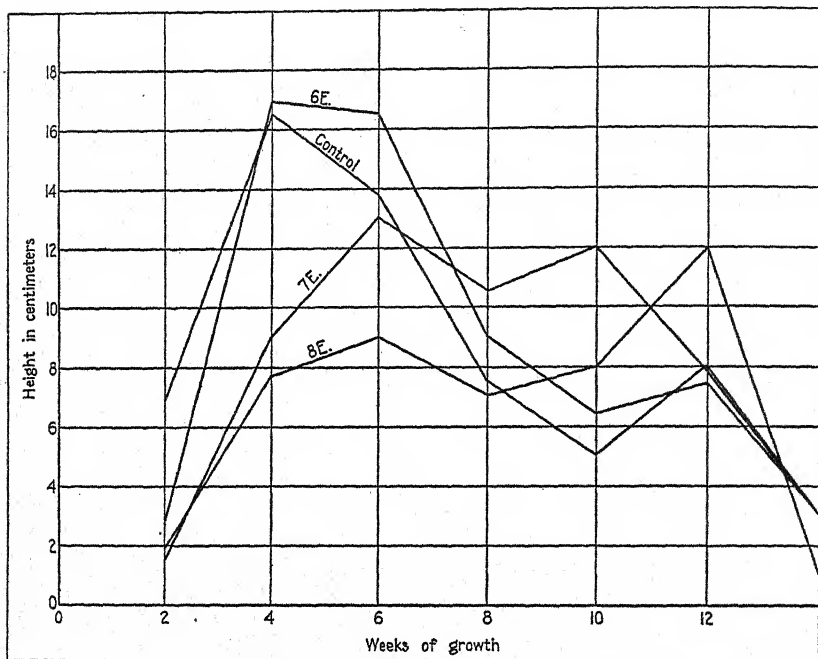
Text figure 2 graphically represents the average total height (measured at weekly intervals) of controls and of plants from three groups of irradiated seeds. It is seen that throughout the life cycles of the irradiated groups there is decreased growth, the ones receiving heavier dosage showing greater depression than those receiving lighter doses. Geller (2) refers to an acceleration of development following an initial checking. That this occurs in seedlings from irradiated seeds is shown graphically by data presented in text figure 3. The polygons represent the weekly increment of growth as computed from data in table 4. During the first three weeks of growth, the weekly increase is approximately inversely proportional to the dose. By the fourth week, however, the irradiated plants show a certain amount of recovery and the weekly increase of growth now approaches or exceeds that made by the controls. The controls made the greatest weekly growth during the fourth and fifth weeks, whereas the plants from seeds irradiated with doses of 6, 7, and 8 *E* showed greatest growth during the sixth, seventh, and twelfth weeks, respectively.

Text figure 4 shows growth curves of plants of the same group as given



TEXT FIG. 3. Diagrams representing weekly increase of growth of controls and plants from seeds irradiated with doses of 6 E, 7, and 8 E, respectively. The greatest amount of weekly growth in the control occurred during the fourth and fifth weeks. The greatest weekly growth of plants from irradiated seeds occurred considerably later. In the case of those receiving the heaviest irradiation, the greatest increase did not occur until the twelfth week.

in text figure 3. The curves for the irradiated plants are of the same general type as that of the control but the highest point of the curve, namely the time when the greatest weekly growth was made, was later in plants from irradiated seeds.



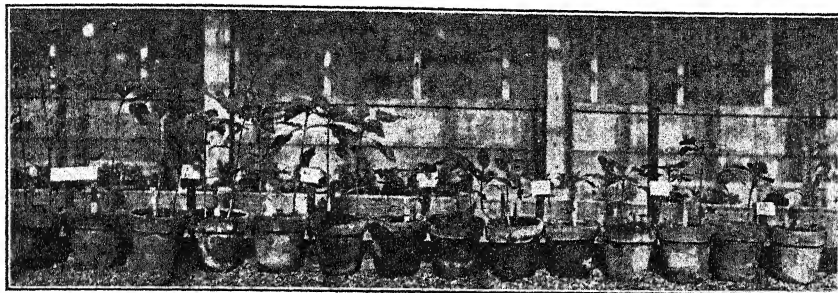
TEXT FIG. 4. Curves showing weekly increment of growth made by controls and by plants from irradiated seeds. The curves of the latter are of the same general type as those of the control, but the greatest weekly increase did not occur until after the period of growth-checking had passed.

Inhibition of growth, practically proportional to the dosage given, is shown in the accompanying photograph of 31-day-old plants from irradiated seeds (text fig. 5). At the age of 53 days, increased growth of irradiated plants shows that they are recovering from the effects of the dosage given the seeds. By the time the flowering stage is reached, they appear of almost equal height to those of the control (text fig. 6). The rapid later growth of the plants, as shown graphically in text figure 3 has caused the series to show little differences in height at maturity and only slight differences in dates of blooming.

This experiment shows that for a period of about three weeks injury seems proportional to the dose given. After that time there is partial recovery from injury and from that time on a more normal growth is made.

A description of the fasciation present in some of the plants of this

series, together with experiments giving effects upon growth when sunflower seedlings were irradiated with doses ranging from 5-10 *E*, has been given in a previous paper by the writer (5).



TEXT FIG. 5. Plants of NS. 2 series showing, during early growth, injury proportional to dose. Two control plants at left and, in order, those whose seeds received doses of 5, 6, 7, 8, 9, and 10 *E*, respectively. Age of plants 31 days.

COMPARISON OF EFFECTS OF THE SAME DOSAGE ON AIR-DRY SEEDS AND SOAKED SEEDS

Seeds in air-dried condition are much less susceptible to the harmful effects of X-rays than are those which have a water content of about 50 percent. This is shown in table 5, which gives the effects of exposing air-dry and soaked seeds to a dosage of 10 *E*. In this table are given also data showing results of irradiating air-dry seeds with 15 *E*. Using height and absolute dry weight as standards by which growth is measured, it seems that a dose of 15 *E* applied to air-dry seeds causes about the same retardation of growth as does a dose of 10 *E* on soaked seeds.

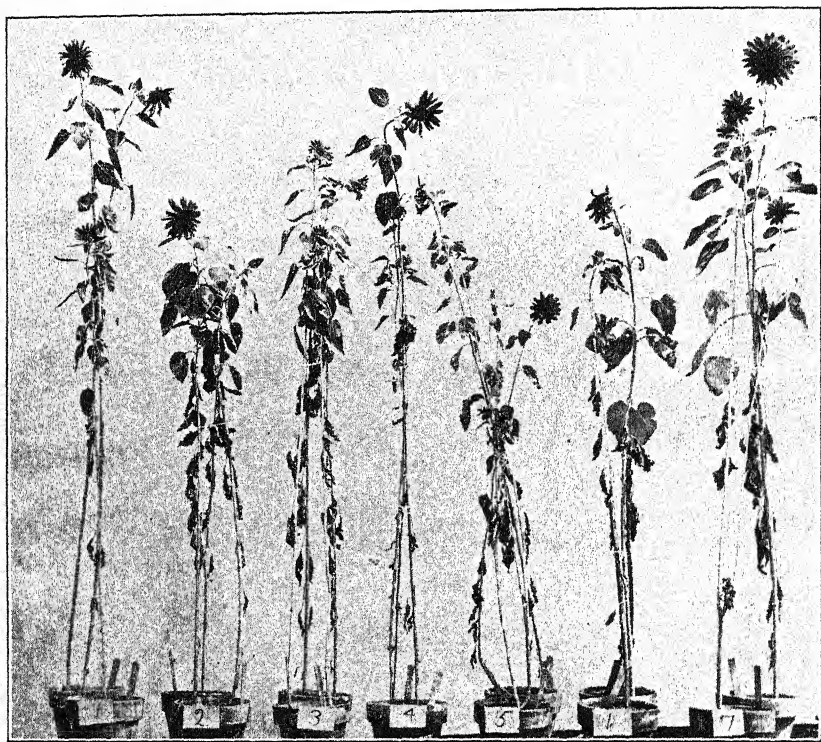
TABLE 5. *Comparison of Effects of Same Dosage of X-rays upon Dry and Soaked Seeds*

Series No.	Seed Condition When Irradiated	Percentage of Germination	Dose	Height in cms. at Time of Blossoming	Total Days of Growth Before Blossoming	Weight in Grams	
						Green	Abs. Dry
IS. 1.....	Air-dry	80	10 <i>E</i>	114	97	111.8	19.12
Control.....	"	80	—	135	95	71.67	12.26
IS. 2.....	56.5% water	75	10 <i>E</i>	83	121	44.24	9.16
Control.....	"	80	—	111	105	81.8	17.21
JS. 1.....	Air-dry	65	15 <i>E</i>	140	109	64.54	13.0
Control.....	"	95	—	171	123	118.71	25.36

It is evident that these experiments confirm those of other investigators who have found that air-dry seeds are less susceptible to X-rays than are soaked seeds.

DIFFERENTIAL INJURY SHOWN BY X-RAYS

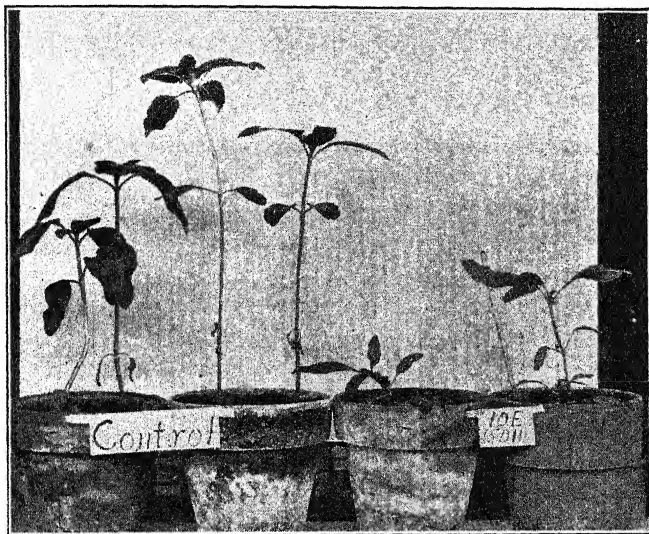
Differential injury due to action of X-rays was strikingly shown in one series of experiments. Similar conditions have been reported by those experimenting with the action on animal tissue and human tissue as well. Mayo (9) states that different tissues in the same person react differently to radiotherapy and that patients with apparently the same degree of cancerous growth may derive different results from the use of X-rays.



TEXT FIG. 6. Plants of NS. 2 series showing, at blooming period, little differences in height and date of blooming. Control plant at left; in order, those from seeds which had received doses of 5, 6, 7, 8, 9, and 10 *E*, respectively. Half of those receiving 10 *E* died. Those surviving are shown on extreme right.

In the experiment described below, soaked seeds were given a dosage of 10 *E*. The heights at blossoming time of the five plants which were carried to maturity were 10 cm., 25 cm., 108 cm., 131 cm., and 142 cm., respectively. The two very small plants were extremely slow in blossoming, taking 12 percent more time to blossom than the controls. This may be explained by the fact that these plants showed injury to such an exceptional degree that the flowering period was greatly delayed. Though blossoming was retarded to such an extent, it is worthy of note that it was not entirely inhibited, even though the plants remained so dwarfed (text fig. 7).

The above facts furnish evidence that even in the same species there is considerable variability among seeds in regard to their susceptibility to X-rays. Both plant and animal tissues are selective to some extent in their reaction to X-rays.



TEXT FIG. 7. Degrees of injury shown by 33-day-old plants whose seeds were given a dose of 10 E. One plant in each of the two pots to the right shows greatly inhibited growth.

SUMMARY

1. Irradiation of sunflower seeds by X-rays does not result in increased rate of germination, increased percentage of germination, increased growth during the early seedling stages, nor in increased height and weight at maturity.

2. When seeds are irradiated with medium doses of X-rays (5-10 E) an inhibitory effect proportional to the dosage is noticeable during the first few weeks of growth. After about three weeks, this growth-checking disappears and the plants grow rapidly until at maturity there is very little difference in total height and in time of blossoming between the controls and the plants from treated seeds.

3. Seeds in air-dried condition are much less susceptible to the harmful effects of X-rays than are those which have a water content above 50 percent.

4. Seeds of the same species show differential injury when exposed to the same dosage and to the same environmental conditions.

The writer wishes to express her thanks to Professor C. A. Shull for suggesting this problem and for his advice and encouragement; to Dr. A. C.

Ivy for facilities offered for irradiating plant material; and to Professor Francis Ramaley for criticism of the manuscript.

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FURTHER EXPERIMENTS IN REPEATED REJUVENATIONS IN HEMP AND THEIR BEARING ON THE GENERAL PROBLEM OF SEX¹

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In 1926 the writer (8) published some observations on rejuvenation in hemp produced by means of continuous illumination. Three successful rejuvenations had been brought about, giving the rejuvenated plants four developmental cycles and four reproductive periods. In the meantime some further work has been done and a number of practical results have been obtained which indicate that a study of repeated rejuvenation in plants can be used in various ways in the solution of both practical and theoretical problems.

In continuation of the previous experiments mentioned above, hemp was planted November 26, 1924, in a large tank about three feet wide and with soil about three feet deep. These plants did not bloom promptly like most winter plants because they received some light at night from electric bulbs employed in another experiment. They were blooming well by March 1, 1925, the extraneous source of light in the meantime having been removed. On May 15 they were given continuous illumination, daylight in the daytime and two 100-watt mazda electric light bulbs at night. Most of the plants rejuvenated and the continuous illumination was maintained during the summer. This kept them in the vegetative condition. The smaller plants were removed to make room for expansion until there were only three staminate and four carpellate plants left in the tank. These were well spaced and had room for indefinite growth.

By September 4 all three of the staminate plants were developing a few staminate flowers but none of the carpellate plants showed signs of blooming. The electric light was turned off September 8, 1925, and by September 22 all the plants were in full bloom. Rejuvenated plants kept in continuous light will, after normal recovery, be brought into bloom in from 8 to 10 days after the night light is discontinued. On this date (September 22), the continuous illumination was resumed and part of the flowers from both the staminate and carpellate plants were removed to prevent too great exhaustion. The plot was also treated with a liberal supply of cow manure. By October 22 all the seven plants were beginning to rejuvenate buds but were still in a weak condition when the room in which the plants were kept was, unfortunately, fumigated with a liberal supply of tobacco smoke. This had a

¹ Papers from the Department of Botany, The Ohio State University, no. 204.

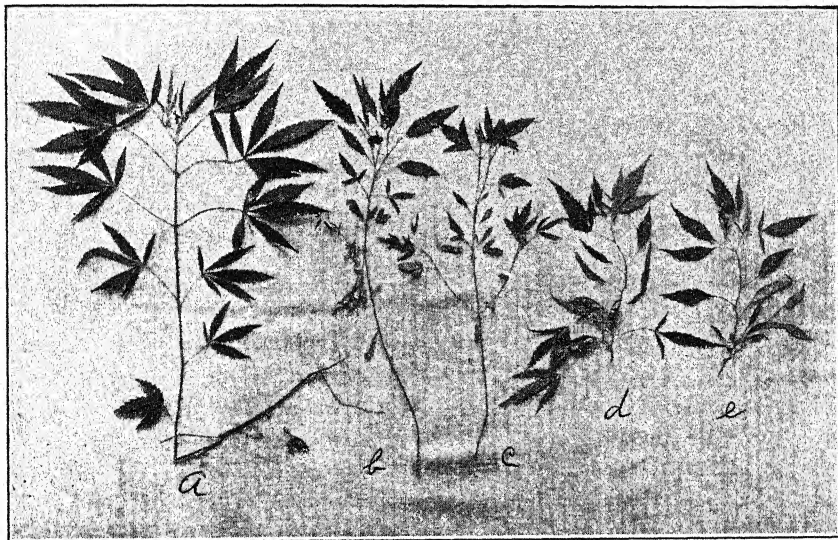
very severe effect on the rejuvenating plants and within two weeks all three of the staminate plants and the weakest individual of the carpellate plants had died. The tobacco fumes were apparently more injurious to the rejuvenating staminate plants than to the rejuvenating carpellate plants. The three remaining carpellate plants finally recovered and continued their rejuvenation growth. These plants continued a normal growth during the winter of 1925-26 and on March 15, 1926 the night light was turned off to induce a third blooming period. In nine days, on March 24, stigmas were protruding from flowers on all three of the plants. Two of the plants now began to show signs of weakness but the third still appeared vigorous and healthy.

At this time and earlier the plants were subject to continuous attacks of scale insects, mealy bugs, and aphids, so that hand cleaning was resorted to but no insecticide was used. The insects attacked these old, rejuvenated plants, while they were entirely absent from vigorous first-growth plants a few feet away. Hand cleaning was necessary two or three times a week to keep the insects in reasonable check. Evidently the senile changes were of a marked character, as compared with the young plants, which caused a definite reaction of some kind in the insects. Morphologically nothing noticeably peculiar distinguished the young plants except that the leaves were alternate, which is the usual condition in rejuvenated shoots.

By March 29 the plants were in full bloom and the electric light was turned on again at night. One of the three plants died on June 2, 1926. It had been weak for some time although it came through the third rejuvenation fairly well and began its fourth vegetative differentiation cycle along with the other two. There were thus two carpellate plants left. They had recovered from their third rejuvenation and were growing well by the first of July, when it became necessary for the writer to leave them. They would probably have survived under the continuous illumination had the insects been eliminated. The last plant died August 4, 1926, the other several days earlier. The two plants were absolutely covered with scale insects. As in former experiments these plants were taken through three successful rejuvenations and four vegetative cycles. Because of the long-continued vegetative periods between the controlled blooming periods, the plants lived for a much longer time than those of the previous rejuvenation experiments. The life of the oldest plant was 1 year, 8 months, and 8 days, counting from the date of planting, or exactly 88 weeks. When hemp is planted in the field in the latter part of May, the staminate plants have usually matured and are dying or dead in about 15 weeks and the carpellate plants survive only a few weeks longer. The rejuvenated plants therefore lived four times as long as their normal life, or more. It is probable that with the elimination of insects several more rejuvenations could be accomplished.

When the plants are allowed to reach an advanced stage of reproduction and senility before each rejuvenation, the leaves produced by the buds, both

terminal and lateral, during the rejuvenation period pass to the simple, entire condition, thus simulating the cotyledons. In many cases the first rejuvenation leaves may still have three leaflets but they are nearly always succeeded by entire, simple leaves (text fig. 1 *a*, *b*, *c*). After each rejuvenation period is completed, the rejuvenated buds undergo a normal differentiation cycle corresponding in general character to the first differentiation cycle beginning with the embryo, except that the leaves usually remain alternate (text fig. 1 *d*, *e*). In some cases, however, a return is made to the opposite-leaved condition. In these cases the rejuvenation is presumably more complete than in the cases where the phyllotaxy remains alternate. A method may perhaps be found to bring the plant back to the normal opposite phyllotaxy after at least the first rejuvenation. In the ordinary complete ontogenetic cycle, rejuvenation is probably accomplished while the cells are in the naked condition, namely, in the sporocyte and gamete phases.



TEXT FIG. 1. *a*, rejuvenated plant, the top of which had died, showing alternate leaf arrangement of the young rejuvenated shoot returned to the normal opposite phyllotaxy *b*, stunted shoot treated at the time of blooming with tobacco fumes and then rejuvenated, showing dead leaves of the first differentiation cycle below, the rejuvenation zone with 3-foliate leaves with entire leaflets, entire simple leaves, serrate simple leaves, and at the tip the beginning of compound leaves again. *c*, same type of plant as *b* but the tip of the main shoot changed back to the normal opposite phyllotaxy. *d* and *e*, branches from plants at the beginning of the third differentiation cycle, showing the second rejuvenation zone. Ripe achenes of the second crop of seed and 3-foliate leaves at the base. On the rejuvenation zone entire simple leaves, followed by serrate simple leaves and at the completely rejuvenated tip by normal, serrate, 3-foliate leaves again.

In the fall of 1926, it was possible to give the rejuvenation method in hemp a practical application. The writer had some hybrid hemp seed from

which he wished to develop the F_1 generation for F_2 generation seed, without danger of contamination from foreign pollen and in time for spring planting in 1927. The seed was, therefore, planted in a room in the greenhouse on September 24. The plants developed rapidly because of the decreasing daylight and began to bloom when quite small. Had they been left to themselves a very insignificant quantity of seed would have been produced. They were, therefore, promptly rejuvenated by means of continuous illumination and all produced numerous branches and developed vigorously. When the light was turned off they bloomed abundantly and a good crop of seed was obtained.

One of the most important applications of the rejuvenation method is in the possibility of having several differentiation cycles in which to gain control of the functional and morphological expression of the individual. This becomes of great theoretical importance in such a problem as sex reversal. It was found that plants which had shown no signs of reversal in their first ontogenetic differentiation cycle could be induced to show it in the rejuvenated cycle. Having seen the disastrous influence of tobacco fumes on old plants undergoing their second rejuvenation, an attempt was made to find out how young carpellate and staminate plants reacted to severe fumigation with tobacco. In the late autumn of 1926, a plot of hemp plants was raised in a shallow tray and when they were come to bloom, so that the sex could be determined without the possibility of a mistake, the tray containing 31 carpellate and 31 staminate plants of about equal vigor was placed in a small compartment and fumigated with tobacco smoke. The effect was very violent on both the staminate and carpellate plants. Most of the leaves were partly or completely killed. After several days they were illuminated at night and on cloudy days with two 100-watt mazda electric light bulbs and supplied with abundance of water. Gradually the staminate plants began to die and although some regained a fair functional activity not one rejuvenated, although under normal conditions the staminate hemp plant can be rejuvenated as readily as the carpellate plant. The staminate plants were all dead by March 24, 1927. Of the 31 carpellate plants, 18 failed to rejuvenate and died, but the remaining 13 began a new cycle of development and showed very characteristic rejuvenation zones (text fig. 1 *b*, *c*). After the plants had passed through the rejuvenation period, three of them were pulled up for preservation as specimens. The remaining 10 were kept in the continuous light and were well watered. All of these plants had a decided lack of photosynthetic surface both while rejuvenating and for some time after because of the injury to the leaves caused by the tobacco fumes. The electric light was turned off on April 15 and all of the plants except a somewhat stunted one promptly came into bloom. The stunted plant was put in a special pot and carefully nursed and soon also began to produce a few flowers. Every one of these 10 surviving carpellate plants showed reversal to maleness and produced some normal staminate flowers. They

were all pure female in expression in their first ontogenetic expression cycle although some might have reversed their sex had they been allowed to become senile before the continuous illumination was supplied. The continuous illumination usually tends, under more normal conditions, to keep the carpellate plants in the pure female state. The 100-percent sex reversal of this group of 10 carpellate plants was probably caused, to some extent at least, by the decided lack of leaf surface during the second growth cycle. The results suggest experiments to induce sex reversal in rejuvenated carpellate plants by the reduction of the leaf surface or otherwise lowering of the metabolism at the proper time, in order to establish a new functional gradient of a different character from the first gradient which followed the sprouting of the seed. Then the secondary female state could be thrown to the secondary male state which would lead on to the primary male state in the male gametes of the pollen grains.

In 1925 the writer (6) reported the complete reversal to maleness of a rejuvenated carpellate hemp plant which had shown only a pure female condition in its normal ontogenetic cycle. It was evident from this result that rejuvenation must be taken into consideration in all speculations on the nature of sexuality whenever such rejuvenation has been shown to be possible. Since in my past experiments on sex reversal in hemp I was never able, with the methods used, to obtain 100-percent reversal, but usually not over 90-94 percent, certain geneticists suggested that the result was due to mixed heredities involving sex factors or factors with various valences; and new formulae were proposed to take the place of the *XX* and *XY* hypothesis of sex inheritance. These new formulae are, however, no nearer the truth than the old ones. The writer (7) as well as Stout (9) has discussed the relation of the differentiation process to sex stability in the individual. Stout in his paper on *Cleome* says that "the morphological differentiations of sex are fundamentally an extension of the phenomena of somatic differentiation." Although it is possible, and perhaps probable, that in the standard variety of hemp, used by the writer, a heterozygous condition of various morphological and physiological hereditary factors for various morphological and physiological characters exists and that some of these factors may actually influence the functional gradients so that there is a greater tendency toward stability on this account in some individuals than in others, nevertheless the new formulae as well as the old are entirely beside the mark because they rest on an improper genetic and ontogenetic analysis of the organism as an active agent. Assume that we have a plot of hemp which has been treated to a given, special environment and the plot in consequence shows 88 percent of carpellate individuals with some degree of reversal to maleness while 12 percent have remained pure in expression; and that 92 percent of the staminate plants show some degree of reversal to femaleness while 8 percent have remained pure. There is still no basis for assuming that the few unreversed carpellate and staminate individuals are

genetically pure for sex or that they are super-males and super-females. Certainly no one who considers the ecological reversal curves which I have obtained would make such a hazardous assumption. Another type of environment might have caused quite a different response in the individuals involved.

Rosa (2) has reported a condition in a certain variety of spinach which he terms *tetramorphic*. Any progeny under the environment employed consists of "extreme males," "vegetative males," "monoecious plants," and "female plants." Besides these an "intergrading vegetative male" is also figured. Rosa also found that "in some strains a considerable portion of the plants, purely pistillate in the early part of their flowering period, produce, later in the season, some staminate flowers toward the tips of the branches, especially of small lateral branches." Just what influence the period of illumination, senility, or other factors may have he did not determine. There is evidently an opportunity for interesting ecological experiments on spinach both on the supposedly "mixed" commercial strains and possible segregated "pure lines" in relation to their sexual behavior. Rosa's conclusion that "the fact that this apparent sex reversion in spinach is limited to certain strains and to certain plants within these strains, while nearby plants under the same conditions continue to be purely pistillate to the end of the season indicates that this apparent sex reversion is connected with potentialities within the plant based on genetic factors" is not at all warranted, and is the same kind of reasoning one finds in general use by most geneticists. Certainly, there are different heredities which show different degrees of stability or instability in a given variety; but the same hereditary constitution in the same general environment will give quite different results, as is shown by the diversity of sexual expression on the different branches of a reversed *Thalictrum* inflorescence, for example, as indicated below. It is evident also that any environmental factor by itself may not be at all efficient when combined with another condition. Thus short photoperiodicity with a comparatively high temperature and the proper substratum is very effective in causing sex reversal in hemp; but a short photoperiodicity with a temperature so low that it will just permit continued growth has no effect whatever on the sexual state of the plant. There is in such a condition no reversal either in the staminate plants or in the carpellate plants. It is the summation of factors, the combined influence, that must be considered. Problems of sex as well as of the expression of hereditary characters in general must be analyzed and solved through a correct ecological conception of the relation which exists between the organism with its hereditary potentialities, on the one hand, and the sum total of internal and external environments on the other. The functional gradients are conditioned by this interaction of the hereditary potentiality and the environment. Genetic theory developed on any other basis is of no ultimate scientific value.

Hirata (1) after experimenting with hemp concluded that the population was made up of the following genetic types:

	Diploid Chromosomes	Haploid Chromosomes
Super-male.....	$\begin{cases} 18 + Y + Y \\ 18 + y + Y \\ 18 + x + Y \end{cases}$	$\begin{matrix} 9 + Y, 9 + Y \\ 9 + y, 9 + Y \\ 9 + x, 9 + Y \end{matrix}$
Male.....	$\begin{cases} 18 + X + Y \\ 18 + x + y \end{cases}$	$\begin{matrix} 9 + X, 9 + Y \\ 9 + x, 9 + y \end{matrix}$
Male intersex.....	$\begin{cases} 18 + y + y \\ 18 + X + y \end{cases}$	$\begin{matrix} 9 + y, 9 + y \\ 9 + X, 9 + y \end{matrix}$
Female intersex.....	$\begin{cases} 18 + x + x \\ 18 + x + X \end{cases}$	$\begin{matrix} 9 + x, 9 + x \\ 9 + x, 9 + X \end{matrix}$
Female.....	$18 + X + X$	$9 + X, 9 + X$

He states that "in the formulae x and y show the X and the Y chromosomes but the x has a higher male tendency than the X and the y has a higher female tendency than the Y but X and Y are not used in those strict morpho-cytological meanings." He says that "both the female and the male factors are contained in the X and the Y chromosome respectively but in the X the valency of the female factor (or factors) is higher than that of the male and in the Y , on the contrary, that of the male factor (or factors) is higher than the female. Thus the X has a net female tendency while the Y has a net male tendency." But it is evident that while such an assumption of genetic categories might seem plausible if we had to judge the individual by a single performance in a single environment, the fact that the rejuvenated individual may express a sex condition indicating an entirely different hereditary constitution from the one first assumed shows that the genetic sex formulae are of no value whatever either in expressing the fundamental conceptions of sexuality or giving reliable prediction of the sex-performance of the individual under changing environmental conditions. Furthermore, we know that the same hereditary constitution can give rise to entirely different gradients and thus a gradation series of expressions of male and female conditions may be produced as in the reversed branches of the inflorescence of dioecious *Thalictrum*. Recently the writer has obtained reversal gradations in the tassel of the monoecious Indian corn.

If genetic labels were to be given to a population of hemp plants which showed an expression series in respect to sex, we may take these very plants, even those labeled "pure," and with rejuvenation and the establishment of a new and different functional gradient cause most of them (and probably all) to reverse to the opposite sex in all degrees of intensity of expression. When geneticists consider the facts of ecological and physiological conditions, we shall have a very different genetics from that which is at present either assumed to be, or is definitely labeled as, "orthodox." The matter of diversity of sexual expression in *Thalictrum* was considered by the writer in 1919 in a paper on some observations on *Thalictrum dasycarpum* (3). In

this paper it was stated that "one can find the same diversity of distribution among the various branches of certain bisporangiate [reversed] individuals as exists among the individuals themselves." Later, in 1923, after some experiments had been performed, it was shown that the sex expression any individual may show in a given period is not due to a peculiar hereditary constitution but to the given environment, and that in another environment in another season it may have a very different expression, corresponding to the peculiar condition shown at the time by its contemporary neighbors (4). Any one considering the evidence presented in this connection can see that with an absolutely identical hereditary complex, the different buds of the large inflorescence produce branches with entirely different functional gradients and thus give rise to a great variety of sex reversals of the same character and degree of difference as can be observed in the individuals of a patch of hemp, treated in the ordinary ways for sex reversal. In the branches of *Thalictrum*, a series will be present ranging all the way from pure types, showing no sex reversal whatever, to branches with very extreme reversal, exactly as the individuals of a patch of reversed hemp can be arranged. If a uniform heredity in respect to sex and other conditions can bring about a diversity of gradients in the branches of a *Thalictrum* inflorescence, there is no necessity and no basis for postulating a series of diverse heredities to explain the diverse expression series exhibited in a progeny like a patch of hemp. Especially is this true since we know that the label that a geneticist might attach to our plants would not at all indicate that we could bring out the same heredity with a second trial in expression, when the plants are rejuvenated and have the opportunity of a new life in which they may behave quite differently from the way they did in the first.

SUMMARY

1. Carpellate hemp plants were rejuvenated by means of continuous illumination three times, thus going through four ontogenetic or expression cycles. At each rejuvenation the cycle of differentiation begins at the same level, the simple entire leaf. Normally the rejuvenated shoots have an alternate phyllotaxy. The most vigorous plants lived 88 weeks which is about four times as long as the normal life of carpellate plants raised out-of-doors.

2. Fumigation by tobacco smoke is more injurious to rejuvenating staminate plants than to rejuvenating carpellate plants. Plants fumigated during the original blooming period showed an apparent difference in resistance although all were greatly injured by the tobacco fumes. Of 31 staminate plants and 31 carpellate plants thus fumigated and then treated for rejuvenation, none of the staminate plants rejuvenated and all but a few began to die rapidly, while 13 of the 31 carpellate plants rejuvenated successfully and the 10 of these which were taken through the second blooming period all showed reversal to maleness in some branches although

at the previous blooming period they were all pure female. This reversal in the second ontogenetic differentiation cycle was probably due to the lack of a proper photosynthetic system because the leaves had mostly been destroyed by the tobacco smoke.

3. The very old, repeatedly rejuvenated plants are especially susceptible to the attack of mealy bugs, scale insects and other forms, which attack the plants severely when nearby first-differentiation-cycle plants show almost complete immunity to such attacks.

4. Rejuvenation was successfully used to induce branching with subsequent increase of seed production on winter-grown hemp, in hybridization experiments.

5. The fact that sex reversal can be successfully brought about during the second ontogenetic differentiation cycle of rejuvenated plants in individuals that were of pure sex expression in the first or natural differentiation cycle indicates that speculations as to the nature and fixity of sex, especially in relation to heredity, are of questionable value, and formulae based arbitrarily on the behavior of individuals during their first differentiation cycle are not to be accepted as an explanation of the real nature of such individuals. Had these individuals been subject to a different environment during their first life, a different functional gradient could have been established with a consequently different character expression in respect to sex.

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A STABLE COLORIMETRIC STANDARD FOR CHLOROPHYLL DETERMINATIONS

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The usual standard used in the colorimetric determination of chlorophyll is a solution resulting from the saponification of the pigment itself. Such a standard has several disadvantages, among which are that: (1) it is unstable; (2) standards prepared from time to time may differ slightly; (3) pure chlorophyll is needed for its preparation, and this is not available to many workers; and (4) those having chlorophyll preparations have no assurance that theirs is of the same purity as those of others.

Owing to its ease of exact duplication and its stability the standard described here does not have these disadvantages. Those desiring to determine chlorophyll but not having the pure pigment should find it especially valuable.

The method for which this standard was devised is essentially that of Willstätter and Stoll² for total chlorophyll. For a standard in this method pure chlorophyll is dissolved in ether, saponified with potassium hydroxid, extracted from the ether with water, and made to volume. For details of the method the original should be consulted.

In searching for a colored solution to replace the standard prepared from chlorophyll itself the idea of using a dye mixture was abandoned, since many dyes are unstable or cannot be obtained in a known state of purity. It was found that a solution consisting of a mixture of copper sulphate, potassium dichromate, and ammonium hydroxid could be used. Solutions of these substances of known concentration can easily be made.

The following solutions were used:

1. Copper sulphate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 10 grams per liter. Crystals covered with the white powder of the less hydrated form should be avoided. If possible, the purity of the salt should be checked.

2. Potassium dichromate, $\text{K}_2\text{Cr}_2\text{O}_7$, 20 grams per liter.

3. Ammonium hydroxid, twice normal.

To make the standard, measure accurately 28.5 cc. of the copper sulphate, 50 cc. of the potassium dichromate, and 10 cc. of the ammonium hydroxid into a 100 cc. volumetric flask; make to volume and mix. When the solution was placed in one cup of the colorimeter and the instrument set at

¹ Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

² Untersuchungen über Chlorophyll, 78-83. Berlin, 1913.

20, 30, or 40 mm. it proved to be colorimetrically equivalent to a solution obtained by saponifying 85 mg. of chlorophyll and making up to one liter.

The green weight percentages of chlorophyll, determined on several leaf samples with both the usual chlorophyll standard and the new standard, are shown as follows:

With Chlorophyll Standard	With New Standard
.305.....	.302
.318.....	.322
.230.....	.230
.187.....	.189
.335.....	.333
.250.....	.248

The author hopes that workers having pure chlorophyll at hand will check the value given as the chlorophyll equivalent of the new standard here described.

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FURTHER STUDIES IN THE RING-SPOT DISEASE OF TOBACCO

C. N. PRIODE

(Received for publication November 25, 1927)¹

In a recent paper² some of the symptoms of the ring-spot disease of tobacco were described and pictured. A careful study of these symptoms led to the belief that ring spot belongs in the virus disease group of plant maladies. The object of the present paper is to report the results of some further studies on the disease. Special attention has been given to symptoms, host range, and means of transmission. Studies have also been made on the keeping qualities of the infectious agent in juices stored at different temperatures.

Although ring spot frequently produces chlorosis which causes mottling, it is essentially an infectious necrosis. On tobacco it attacks leaf tissues only. No visible symptoms ever appear on stems or on large leaf veins. It is typically a leaf-spot disease. Chlorotic and necrotic spots occur in the form of small circular areas, rings with green centers, concentric circles, or wavy lines which follow along the leaf mid-ribs and leaf veins. The tissues composing the spots commonly die. In some cases, however, they live indefinitely but are more or less chlorotic.

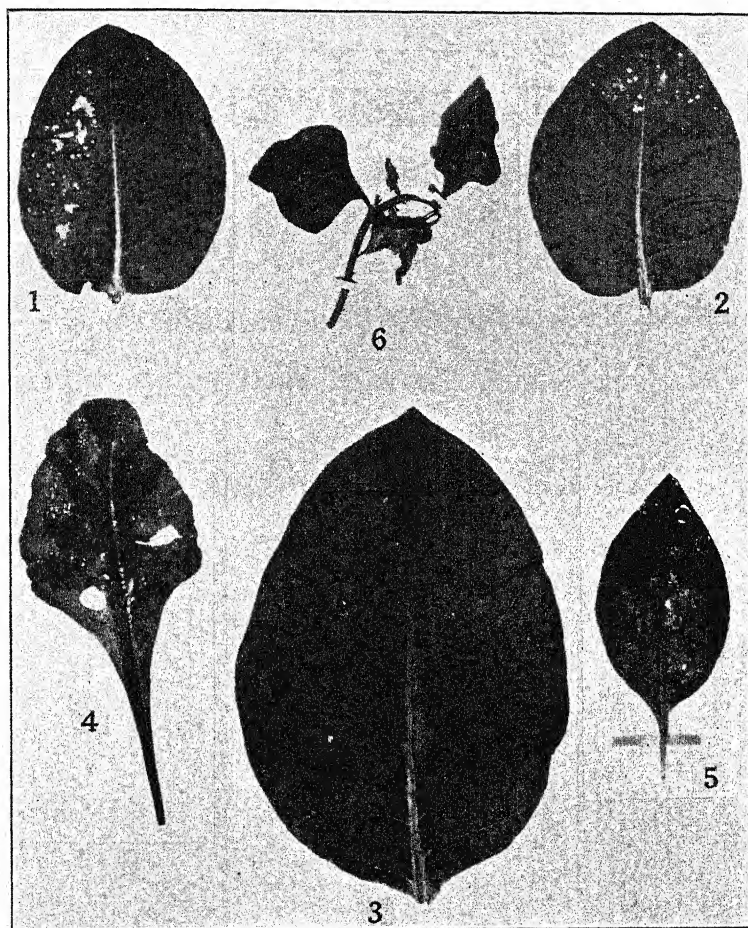
Ring spot is a systemic disease. Lesions usually appear on all or most of the leaves above the point of inoculation. It differs from most virus diseases, however, in that the first symptoms appear at or near the point where a leaf is inoculated. Systemic infection occurs a few days after the first symptoms of local infection appear.

Ring-spot disease has been produced repeatedly on definite portions of leaves by applying inoculum to those portions. In one experiment thirty plants were inoculated. One leaf on each of ten plants was inoculated by applying plant juice containing the infectious agent to portions of the blade on one side of the mid-rib. Within four days rings developed on the inoculated portions. Uninoculated portions of these leaves remained healthy, as shown in text figure 1. Ten of the plants were inoculated by applying infectious juice to the basal portions of a leaf on each plant. After four days rings developed on the inoculated areas but did not appear on other parts of the inoculated leaves. The other ten plants were inoculated by applying infectious juice to the tip portions of their leaves. Here

¹ Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

² Fromme, F. D., S. A. Wingard, and C. N. Priode. Ringspot of tobacco; an infectious disease of unknown cause. *Phytopath.* 17: 321-328. 1927.

again rings appeared on the areas inoculated but did not appear on uninoculated parts, as is shown in text figure 2. Five days after the development of local symptoms, rings appeared on the young leaves of all of the thirty plants. In every case local infection was followed by systemic infection. In other experiments a few instances were observed where systemic infection failed to follow local infection. In such cases the disease occurs in inoculated tissues only. If inoculations are made into stem tissues systemic infection occurs without the production of local symptoms.



TEXT FIG. 1. Rings produced on half of tobacco leaf to which inoculum was applied. TEXT FIG. 2. Rings produced on tip portion of tobacco leaf where inoculum was applied. TEXT FIG. 3. Tobacco leaf showing rings formed around needle-puncture inoculations. Two uninoculated needle-puncture wounds are shown on the side opposite the ring spots. TEXT FIG. 4. Ring spot on a beet leaf. TEXT FIG. 5. Ring spot on a leaf of *Phytolacca decandra*. TEXT FIG. 6. Wilt produced by ring spot on New Zealand spinach.

Other experiments were performed which gave further proof of the ability of the disease to produce local infection at the point of inoculation. The pointed end of a needle was wrapped with a small bit of absorbent cotton. The cotton was then saturated with infectious juice. One hundred and twenty needle-prick inoculations were made into the leaves of healthy plants. Enough pressure was used to force some of the juice from the cotton into each of the needle-prick wounds. Similar uninoculated needle-prick wounds in the same leaves served as checks. Six days after the inoculations were made, rings had formed around thirty-six of the one hundred and twenty needle-puncture inoculations. No infections resulted apparently from the other eighty-four inoculations or from the check needle-puncture wounds. Text figure 3 shows some of the rings and their relation to the needle wounds. In most instances the rings formed concentrically around the needle punctures. In some cases, however, they developed eccentrically. Each successful inoculation produced a single ring spot with its center at or near the point where the needle jab was made.

Although ring spot occurs abundantly on tobacco in the field it has never been observed on other species of plants. The ease of mechanical transmission of ring spot from diseased to healthy tobacco plants suggested the possibility that it might be transmitted to other species. Accordingly about 20 different species, including potato, tomato, pepper, and eggplant, were inoculated with juice from diseased tobacco plants. The disease was successfully transmitted to only four of the plants subjected to the test. The plants found to be susceptible are the beet (*Beta vulgaris* L.), pokeweed (*Phytolacca decandra* L.), petunia (*Petunia hybrida*) and New Zealand spinach (*Tetragonia expansa* Murr.). Beet and pokeweed plants developed typical rings on the leaves to which the inoculum was applied. No symptoms appeared elsewhere on the plants. Systemic infection was not obtained. A diseased beet leaf is shown in text figure 4 and a diseased pokeweed leaf in text figure 5. Petunia plants proved to be very susceptible to the disease. Symptoms on petunia were similar to those on tobacco. Ring spots appeared first on the portions of leaves to which inoculum was applied. Three to four days after the appearance of local symptoms systemic infection appeared on the young leaves at the tips of the branches. Young petunia leaves show chlorosis rather than necrosis during early stages in the development of the disease. The chlorotic areas consist of wavy lines and rings. After a few days most of the affected tissues die and collapse but some of the chlorotic rings remain alive indefinitely. Ring spots similar to those observed on tobacco leaves were produced on full-grown petunia leaves by means of needle-prick inoculations. In one experiment rings developed around twenty-seven of forty-five needle-prick wound inoculations.

The symptoms of ring spot on New Zealand spinach are similar to those on tobacco and petunia. Unlike any of the other hosts, however, New Zealand spinach shows stem symptoms. These appear first as small,

discolored, depressed streaks or elliptical ring spots near the points at which diseased leaves are borne. As the disease develops the streaks and rings become more pronounced and extend to all upper portions of diseased stems. Necrosis in the stem tissues of this plant is so severe that the tips of some branches wilt and die, as is shown in text figure 6. Microscopic examination of free-hand sections of diseased stems shows necrosis and blackening of affected tissues. No trace of bacteria or fungi could be found in the stem lesions. Young tobacco plants inoculated with juice from diseased New Zealand spinach stems developed typical ring-spot symptoms after five days.

It is well known that the infective principle of the common tobacco mosaic is retained in dried leaves for long periods of time. The infective agent of ring spot apparently loses its virulence after a short period of drying in diseased leaves. Tobacco leaves affected with ring spot were harvested and divided into two portions. One lot was hung up in a greenhouse to dry; the other was allowed to dry in a laboratory where the light was less intense. After one month the leaves of each lot were ground to a fine powder. The powder was placed in a small amount of water in each of two flasks. After thirty minutes of soaking, material from each lot was used to inoculate eight young tobacco plants. All of the plants remained healthy. Similar experiments with juice expressed from diseased plants were undertaken. It was found that the causal agent in ring-spot juice quickly loses its virulence when stored at or above room temperature. At lower temperatures the virus retains its virulence for a much longer period of time. On April 1, 1927, six flasks, containing a small quantity of juice from diseased leaves, were stored at the following temperatures: -5°C. , 0°C. , 5°C. , 10°C. , 15°C. , and 20°C. Young tobacco plants were inoculated with juice from each of the flasks one day after they were stored and at different intervals thereafter as shown in table 1. It will be seen that the only sample of virus that retained its virulence over the period of 85 days during which the experiment was in progress was that held at -5°C. The sample held at 0°C. lost its virulence after about three weeks; that held at 20°C. after one day; that held at 15°C. after four days; that held at 10°C. after 12 days, and that held at 5°C. after twenty days. The experiment shows that the length of time during which the virus retains its virulence in expressed juice varies inversely with the temperature at which the juice is stored.

The above experiments suggest that ring spot is not carried over winter in dead tissues. It was thought that the disease might be transmitted through seeds. In order to test this, six hundred tobacco plants were grown to maturity from seeds taken from badly diseased plants. All of these plants remained healthy, indicating that the disease is not readily transmitted through seeds.

Many of the characteristics of ring spot indicate that it belongs in the

TABLE 1. *Effect of Temperature on the Inactivation of the Ring-spot Virus When Stored*

No. Days Stored	Temperature at Which Juice Was Stored											
	- 5° C.		0° C.		5° C.		10° C.		15° C.		20° C.	
	No. Plants Inocu- lated	No. Plants In- fected	No. Plants Inocu- lated	No. Plants In- fected	No. Plants Inocu- lated	No. Plants In- fected	No. Plants Inocu- lated	No. Plants In- fected	No. Plants Inocu- lated	No. Plants In- fected	No. Plants Inocu- lated	No. Plants In- fected
1	5	5	5	3	5	3	5	3	5	1	5	0
3	5	5	5	4	5	4	5	3	5	0	5	0
7	4	3	4	4	4	3	4	2	4	0	4	0
11	4	3	4	3	4	2	4	0	4	0		
15	4	4	4	3	4	2	4	0	4	0		
17	4	4	4	1	4	1	4	0				
19	5	5	5	1	5	0						
21	5	4	5	0	5	0						
23	5	4	5	0								
29	4	4										
35	4	4										
44	4	4										
60	4	3										
75	4	4										
85	4	4										

virus disease group. Since the agents causing most of these diseases are filter-passers, an effort was made to determine whether or not the virus of ring spot is filterable. A portion of juice extracted from diseased leaves by grinding and pressing was passed through a Berkfeldt filter of grade "N" and inoculated into each of ten healthy young tobacco plants. At the same time an unfiltered portion of the same juice was inoculated into four similar healthy young tobacco plants. After four days the plants inoculated with unfiltered juice became diseased. The ten plants inoculated with filtered juice remained healthy. This experiment was repeated with another sample of virus-bearing juice. The disease was not obtained by inoculating with filtered juice. Whether the agent causing ring spot will pass filters of coarser grade than "N" has not been determined. Although the infective agent did not pass the filters used, ring spot is nevertheless believed to belong in the virus group of plant diseases.

SUMMARY

1. Ring spot produces both localized and systemic infections. Local symptoms appear 3-5 days after inoculation. Systemic infection develops a few days later.
2. Rings were produced around needle-prick inoculations on both tobacco and petunia plants.
3. Ring-spot infection was produced on four hosts other than tobacco: beet, pokeweed, petunia, and New Zealand spinach. Unlike the other hosts, New Zealand spinach developed symptoms on the stems.

4. No infection was produced from dried diseased material.
5. Juice stored at -5°C . produced infection when applied to plants 85 days after extraction. When juice is stored at higher temperatures, virulence is lost very rapidly.
6. The infectious principle in juice from ring-spot tobacco plants does not pass a Berkfeldt filter of grade "N."

The writer is indebted to Dr. L. O. Kunkel for valuable suggestions and advice during the progress of this work, and for a careful reading and criticism of the manuscript.

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MULTIPLICATION OF THE VIRUS OF TOBACCO MOSAIC IN DETACHED LEAVES

HELEN A. PURDY

(Received for publication November 25, 1927)¹

INTRODUCTION

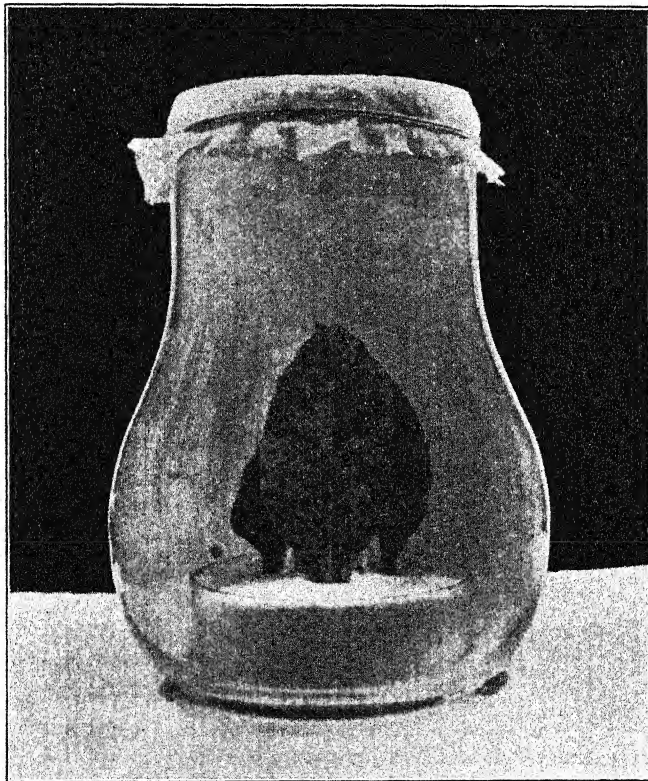
When a tobacco plant takes mosaic disease, the first macroscopic evidence of infection is a clearing of the veins followed by a mottling of the youngest leaves of the shoot. Any immature leaf younger than the one inoculated may exhibit this symptom, but a leaf that is fully formed at the time the virus is introduced into the plant never becomes mottled. The correlation existing between age of leaves and the most characteristic external manifestation of mosaic disease suggests that multiplication of the virus may require the presence of meristematic tissue. But as Allard (1) has demonstrated, all leaves of an affected plant, irrespective of mottling, contain infectious sap. If we assume that the virus is capable of multiplication only in formative tissues, there must be a subsequent distribution from these localized areas.

It seemed likely that some pertinent information might be obtained through an effort to multiply the active principle of mosaic disease in leaves detached from tobacco plants just prior to inoculation. Accordingly, virus was introduced into a number of detached leaves and, after a suitable incubation period, intracellular bodies were observed similar to those described in mosaic tobacco by Iwanowski (5), Goldstein (3, 4), Rawlins and Johnson (7), and others. The presence of these cell inclusions was interpreted as evidence of infection. Moreover, the sap expressed from these leaves, after dilution far beyond the point required to inactivate the virus originally introduced (2), produced one-hundred-percent mosaic disease when inoculated into healthy tobacco plants. The results of these preliminary tests plainly indicated that a multiplication of the virus takes place in the detached leaves. To confirm this finding, serial transmission experiments were undertaken (6) whereby the virus introduced into the first detached leaf of each series would soon become inactivated by high dilution in the course of transmission from leaf to leaf. Therefore, unless the virus multiplied, sap from detached leaves receiving the original inoculum only in high dilution would not be infectious.

¹ Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

METHODS

Leaves varying in length from 9.9 to 21.4 cm. were detached from healthy tobacco plants and inoculated by rubbing 0.01 or 0.02 cc. of undiluted sap from mosaic tobacco leaves into a scarified area at the tip of each leaf. Following insertion in moist quartz sand, the inoculated leaves were placed under lantern globes and kept in a laboratory for a period of incubation varying from 7 to 24 days. The lantern globe was used to exclude insects and also to produce a moist atmosphere. Text figure 1 shows the way in which leaves were incubated while the experiment was in progress.



TEXT FIG. 1. Method of incubating leaves.

The sand was moistened daily with distilled water. In order to ascertain whether or not any appreciable growth takes place in the detached leaves during incubation, a number of leaves were measured from base to tip along the mid-rib at the time of inoculation with virus and again at the end of the period of incubation. The leaves were then examined for the presence of cell inclusions commonly associated with tobacco mosaic. A small piece of epidermal tissue was stripped from the back of a mid-rib or vein and mounted

in a 0.5-percent aqueous solution of iodine green. After a few moments, the host nuclei appeared turquoise blue while the intracellular bodies became pinkish.² No attempt was made to find the inclusions in all of the detached leaves studied. They were found in enough instances to show that they are commonly produced in affected detached leaves. At the end of the incubation period, the sap was expressed from each detached leaf and 1 cc. of distilled water was added in every case. Either 0.01 or 0.02 cc. of this diluted sap was then inoculated into a second detached leaf. The entire process was repeated through eleven series, in six of which ten leaves were inoculated successively. In order to increase the possibility of transmission of the virus in every series, several detached leaves were inoculated with sap from the same leaf of a given series. By this method, it was possible to estimate the approximate amount of virus introduced into each detached leaf of every series, assuming that no multiplication had occurred.

The infectiousness of the sap expressed from the detached leaves was determined in every case by its ability to induce mosaic disease in tobacco or tomato plants.

The virus employed for inoculating the first leaves of the different series was obtained from two sources, but the results in each case were similar. Tobacco leaves affected with a common field mosaic, kindly supplied by Doctor James Johnson, were taken as the source of virus for seven of the series, while mosaic leaves from a tobacco plant growing in a greenhouse at the Boyce Thompson Institute were employed for the four remaining series. Throughout the experiments every precaution was taken to avoid accidental transmission of virus. Additional leaves were taken as controls from all plants used as a source of detached leaves. Sap from a healthy plant was substituted for virus as a source of inoculum, otherwise the experiments described above for serial transmission of the virus were carefully duplicated. It is evident that the control leaves would serve as a check in the event that leaves were detached from a plant affected with mosaic disease in its incipient stages, for in such a case sap from the control leaf would induce infection in the inoculated control plants.

The total number of leaves included in the serial transmission experiments in which virus was used is given in table 2. Of the 171 leaves inoculated, the sap from 146 caused mosaic disease in tobacco and tomato plants, indicating a successful transmission of virus in the majority of cases.

The dilution of the original inoculum introduced into every detached leaf of each series was computed. The number of leaves inoculated with a given dilution is reported in table 3 with the results obtained from testing the infectivity of the sap expressed from each of these leaves. Of 142 detached leaves inoculated with dilutions of the original inoculum exceeding

² This contrast staining reaction is not permanent but lasts only a few hours. This stain was suggested to me by Doctor Francis O. Holmes as a useful means of differentiation between the host nucleus and the intracellular body.

2×10^{-5} , 120 contained sap capable of inducing mosaic disease in tobacco and tomato plants.

RESULTS

The results obtained from attempts to transmit virus through eleven individual series of detached leaves are recorded in table 1.

TABLE 1. *Transmission of the Virus in Individual Series*

Detached Leaf Inoculated	No. of Individual Series Included	No. of Leaves Containing:	
		Infectious Sap	Non-infectious Sap
1st leaf of series	11	10	1
2d " " "	10	9	1
3d " " "	9 *	7	1
4th " " "	7	7	0
5th " " "	7	7	0
6th " " "	7	7	0
7th " " "	7	6	1
8th " " "	6	6	0
9th " " "	6	6	0
10th " " "	6	6	0
Total	75	71	4

* Sap from the corresponding control leaf in one case also proved infectious; therefore the series was discontinued and the results excluded.

TABLE 2. *Results of Inoculations of All Leaves of Each Series*

Detached Leaf Inoculated	No. Detached Leaves Inoculated	No. of Leaves Containing:	
		Infectious Sap	Non-infectious Sap
1st leaf of series	11	10	1
2d " " "	10	9	1
3d " " "	9 *	7	1
4th " " "	10 †	10	0
5th " " "	24	22	2
6th " " "	25	22	3
7th " " "	18	17	1
8th " " "	17	12	5
9th " " "	18	16	2
10th " " "	30	21	9
Total	171	146	25

* Cf. footnote to table 1.

† In order to increase the possibility of transmission of the virus in every series, several detached leaves were inoculated with sap from the same leaf of a given series.

Forty-one detached leaves in which virus multiplied showed an average increase in length during incubation of only 1.58 cm., or 9 percent of the average length of the leaves. In one case, a leaf increased 14 percent of its length, while the minimum elongation observed was about 0.6 percent.

TABLE 3. *Results of Inoculations of Detached Leaves With Various Dilutions of Virus*

Dilution of Original Inoculum	No. Detached Leaves Inoculated *	No. of Leaves Containing:	
		Infectious Sap	Non-infectious Sap
Undiluted 0.01 cc.	4	4	0
Undiluted 0.02 cc.	7	6	1
2 x 10 ⁻²	5	5	0
1 x 10 ⁻²	5	4	1
4 x 10 ⁻⁴	4	3	1
2 x 10 ⁻⁴	3	3	0
1 x 10 ⁻⁴	1	1	0
8 x 10 ⁻⁶	3	3	0
4 x 10 ⁻⁶	4	4	0
2 x 10 ⁻⁶	3	3	0
16 x 10 ⁻⁸	7	7	0
8 x 10 ⁻⁸	10	8	2
4 x 10 ⁻⁸	7	7	0
32 x 10 ⁻¹⁰	7	6	1
16 x 10 ⁻¹⁰	11	9	2
8 x 10 ⁻¹⁰	7	7	0
64 x 10 ⁻¹²	2	2	0
32 x 10 ⁻¹²	11	10	1
16 x 10 ⁻¹²	3	3	0
8 x 10 ⁻¹²	2	2	0
64 x 10 ⁻¹⁴	6	3	3
32 x 10 ⁻¹⁴	6	4	2
16 x 10 ⁻¹⁴	5	5	0
128 x 10 ⁻¹⁶	7	7	0
64 x 10 ⁻¹⁶	4	3	1
32 x 10 ⁻¹⁶	7	6	1
256 x 10 ⁻¹⁸	9	9	0
128 x 10 ⁻¹⁸	9	7	2
64 x 10 ⁻¹⁸	12	5	7
Total	171	146	25

* Cf. footnote to table 1.

No mottling of the detached leaves ever occurred. Those in which virus multiplied were macroscopically indistinguishable from the corresponding control leaves.

DISCUSSION

There were doubtless some dividing cells present in the detached leaves during the incubation period. Probably after detachment from the plant, meristematic tissues are produced in some of the leaves. It was observed that when detached leaves were allowed to remain in the quartz sand for four or five weeks, they developed roots. But considering the brief incubation period of one week, which was adequate for multiplication of the virus, active growth of the detached leaves was reduced to a minimum.

It will be noted that the dilution of the 0.01 or 0.02 cc. of inoculum introduced into the first leaf of each series attained through serial transmission is estimated quite conservatively, since no account is taken of the sap present in the inoculated leaf, which varied in amount from approximately 0.5 to 1.5 cc. Also it was assumed that all of the inoculum intro-

duced was recovered from each macerated leaf, whereas only part of the sap actually was expressed. Consequently, the dilutions of original inoculum recorded in table 3 as capable of inducing mosaic disease in plants after incubation in a detached leaf are considerably underestimated.

SUMMARY

Leaves were detached from healthy tobacco plants and inoculated with the virus of tobacco mosaic. After a suitable incubation period, characteristic intracellular bodies were found in many of the detached leaves, although no mottling nor other macroscopic symptom of disease was apparent. The presence of these cell inclusions was interpreted as evidence of infection.

It was then shown by serial transmission experiments that the causal agent of tobacco mosaic multiplies in the detached leaves. The presence or absence of virus in each detached leaf was determined by the ability of the extracted sap to induce mosaic disease in healthy tobacco or tomato plants.

The author wishes to express her gratitude to Doctor L. O. Kunkel for suggestions during the course of these experiments and for a critical review of the manuscript.

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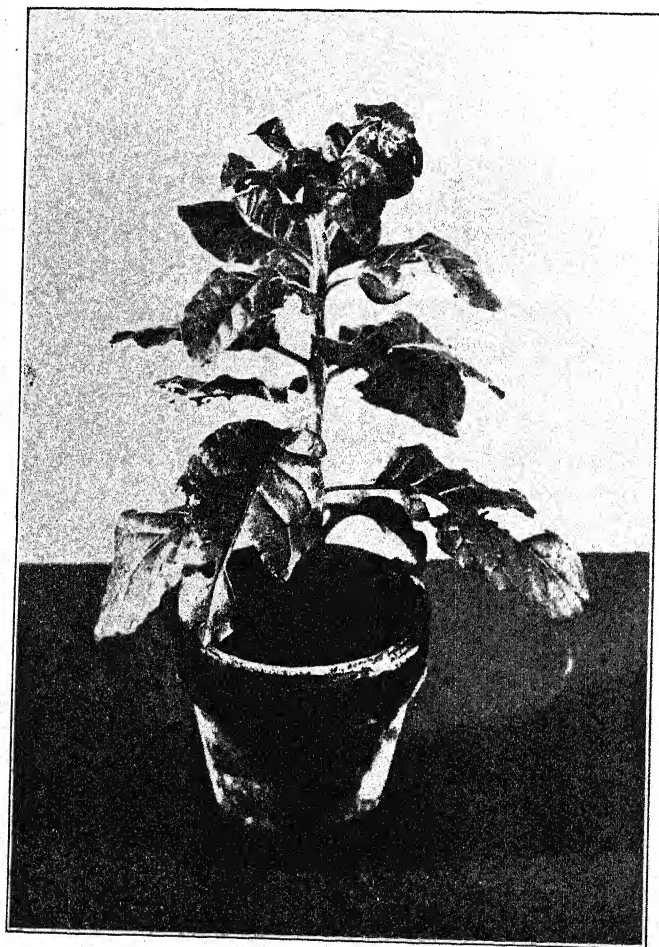
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THE IMPROBABILITY OF TOBACCO MOSAIC TRANSMISSION BY SLUGS

HELEN A. PURDY

(Received for publication November 25, 1927)¹

During an infestation of slugs in greenhouses among tobacco and tomato plants used for the study of mosaic disease, the question arose as to whether or not these pests could transmit the disease. Experiments were carried out in which slugs that had fed upon tobacco and tomato plants affected with mosaic disease were transferred to healthy tobacco and tomato plants and allowed to feed heavily upon them.



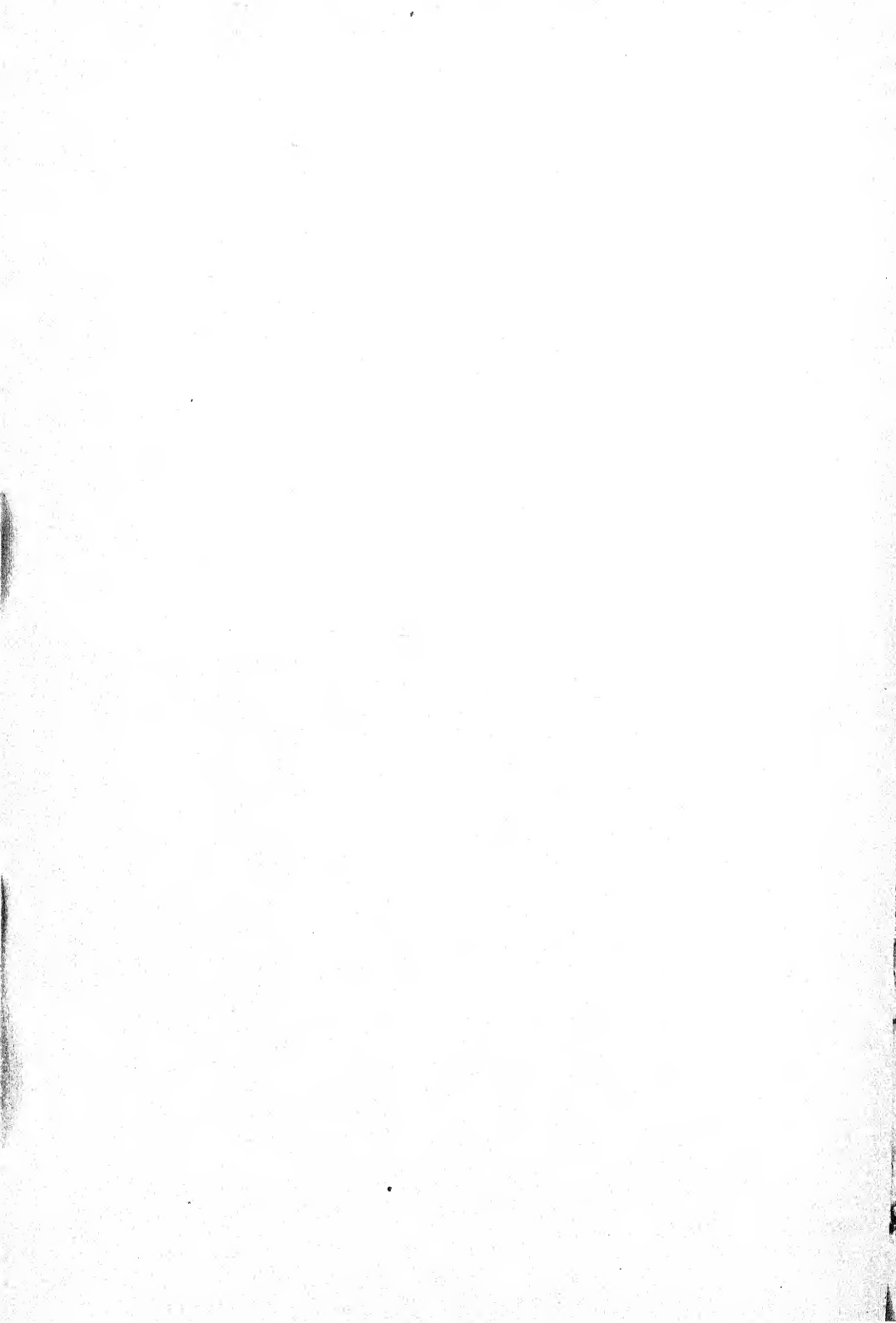
TEXT FIG. 1. Healthy tobacco plant upon which infested slugs have fed.

¹ Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

The slugs were confined under bell jars upon diseased plants for two to fourteen days. They were then transferred to healthy plants for periods varying from one to twenty-seven days. Two species of slugs were used in these experiments, *Limax maximus* and *L. agrestis*, kindly identified by Doctor Roy Miner of the American Museum of Natural History. The slugs were not placed directly upon the leaves of a plant but were allowed to crawl up to the plant from the base of the pot, as they would pass naturally from one plant to another in the greenhouse experiments. From three to eight slugs were transferred from a diseased to a single healthy plant. Text figure 1 shows a healthy tobacco plant upon which slugs from a diseased plant were allowed to feed. The extent to which the slugs fed is shown. The distortion at the tip of the shoot is the result of crowding in a lantern-globe cage.

Twenty-three attempts were made to transmit mosaic disease through slugs by transferring them after feeding upon diseased plants directly to healthy ones. *Limax maximus* was used in all but three of the cases. In eleven of the twenty-three attempts the slugs were transferred successively through series comprising two to five healthy plants, allowing one or two days for feeding upon each healthy plant. A total of fifty-one plants were tested for transmission of tobacco mosaic by this method and not a single case of infection resulted. However, when slugs that had fed upon mosaic material were macerated, they were found to contain bits of green plant tissue which, when inoculated into healthy plants, readily produced infection.

The results of these experiments indicate that transmission of mosaic disease among tobacco and tomato plants in greenhouses through the agency of slugs may be regarded as very improbable.



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No. 2

STUDIES ON THE GROWTH OF ROOT HAIRS IN SOLUTIONS V. ROOT-HAIR ELONGATION AS AN INDEX OF ROOT DEVELOPMENT¹

CLIFFORD H. FARR

(Received for publication January 6, 1927)

In the first paper (I) of this series a description of the author's method of investigating root hairs and a summary of previous knowledge as to their composition, structure, and behavior was presented. In the next two papers (II and III) the effects of different concentrations of three substances, namely, the nitrate, chlorid, and hydroxid of calcium, upon the rate of their elongation were compared. In the preceding paper (IV) there were presented the results of an intensive study of the effects of molar and hydrogen-ion concentrations upon the growth rate of these root hairs in calcium chlorid solutions; and it was shown that on the pH scale certain critical points, namely, the median minimum, the alkaline optimum, the acid optimum, the alkaline limit, and the acid limit, vary with the molar concentration of the salt. In view of the significance of this work as contributing to our knowledge of the relation of plants to their culture media, including the soil, it becomes highly important that some attempt be made to discover whether or not the situation which obtains for root hairs applies also, to some extent at least, to the development of the entire root system, and therefore, presumably, to the entire plant.

In addition to the measurements of root-hair elongation already reported, observations were made upon certain features of the root and root hairs twelve hours after immersion. These included the approximate average spacing, the form, the distribution, and the diameter of amphibious and aquatic root hairs, respectively; the maximum length of aquatic root hairs; the curvature of the root, if any; the length of the zone of aquatic root hairs; the length of the hairless portion of the root tip; the length of the zone between the aquatic and the amphibious root hairs (interzone); and

¹ This paper is the fifth of a series of six appearing in successive issues of the Journal.

[The Journal for January (15: 1-101) was issued February 11, 1928.]

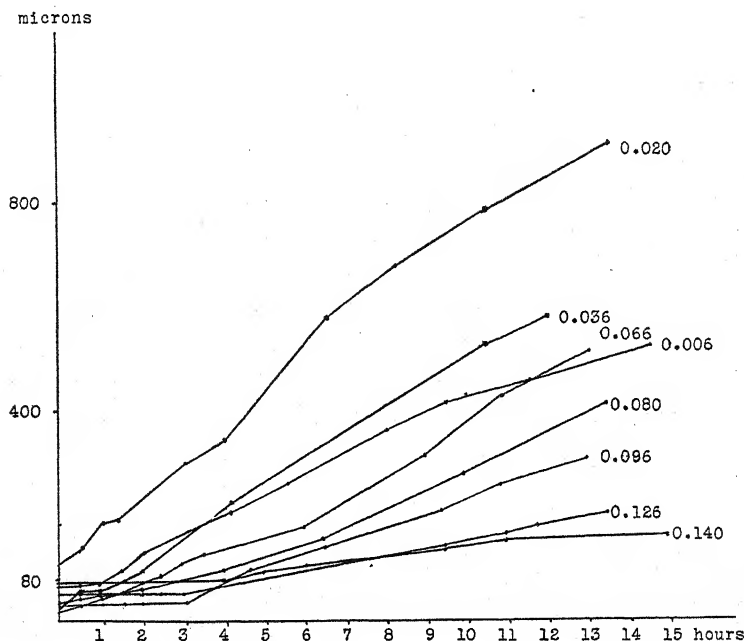
modifications of the form of the root and of the root hairs. The modifications in form and curvature of the roots and root hairs will be presented in the next paper (VI) of the series.

At first it may appear that many of the features referred to above are strictly related to the root hair and not to the root. However, the spacing and the distribution of root hairs, as well as, perhaps, their diameter, may be looked upon as fundamentally responses of root activity. Even the rate of elongation of the root hair has been correlated with root phenomena. Mer (177) in 1879 suggested that a reduction in the longitudinal growth of the root is necessary to increase root-hair formation. Thus he states that increased humidity favors root growth but inhibits root-hair formation. He gives a like explanation of the fact that root hairs are not commonly formed in aqueous media. Schwarz (31), however, arrived at the opposite conclusion, namely, that the most root hairs are formed when the root grows the fastest. Miss Snow (33) and Jeffs (13) noted that as the rate of elongation of root hairs in a damp atmosphere accelerates during the first one or two hours of their development, they are being carried along by the elongation of the root at a slower and slower rate. Thus as the superficial root cell is retarded in elongating vertically, it accelerates its elongation horizontally, resulting in the increase in cell volume being approximately constant. No such lateral movement of the root hairs occurs in aqueous solutions; but its existence in a damp atmosphere indicates that the enlargement of the root hair may not be a fundamentally different phenomenon from the enlargement of other tissue cells of the root.

The maximum length of aquatic root hairs after the root has been immersed for 12 hours does not necessarily have a direct relation to the rate of elongation, although in a solution supporting a rapid growth it would be expected that the maximum length would be greater. The difference between the two sets of data lies in the existence in most solutions of a latent period just after immersion, during which no root-hair elongation occurs. The occurrence of such a latent period is difficult to demonstrate in the case of aquatic root hairs, inasmuch as they do not start to develop until after immersion. Amphibious root hairs, however, show it very plainly; and its existence in aquatic root hairs is indicated by the presence of an interzone in some solutions, which evidently means that the root after immersion has continued to elongate for a time without producing any root hairs.

The lengths of amphibious root hairs at successive intervals after immersion in various solutions is shown in text figure 11. In the three solutions supporting the most rapid growth and producing the longest hairs after the lapse of 12 to 13 hours, no latent period is detected immediately after immersion, but the root hairs continue to grow at approximately the same rate as before. In the other five solutions (0.006, 0.080, 0.096, 0.126, 0.140 *M* CaCl_2), the weakest and the four most concentrated solutions,

there is a latent period following immersion varying from one to three hours in length, during which no growth of root hairs, which formerly had been growing in air, occurred. It thus appears that except in solutions of



TEXT FIG. 11. Lengths of amphibious root hairs at successive intervals after immersion in calcium chlorid.

approximately optimum molar concentration there is a temporary cessation of growth of root hairs after immersion. The observations made as to the occurrence of an interzone, reported below, further indicate that a similar latent period occurs in all hydrogen-ion concentrations which are not close to the optimum, except that in very acid solutions there is a tendency for the root hairs to grow at once, if at all.

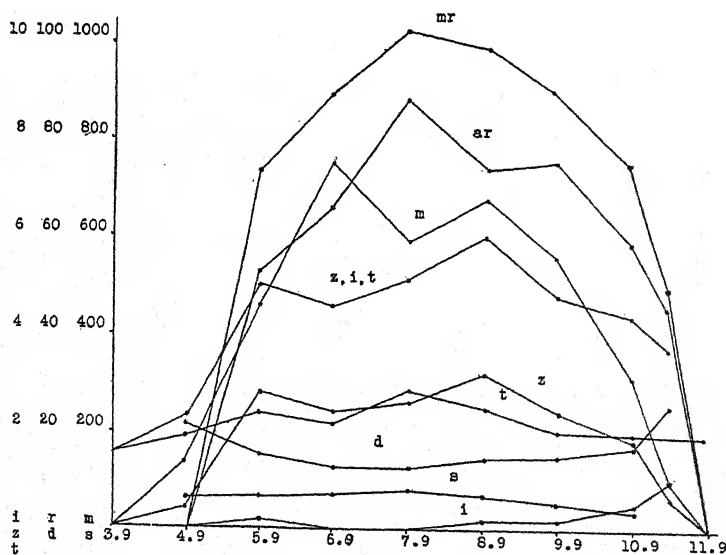
Some of the features referred to above can be measured rather accurately and the results reduced to a basis of mathematical comparison. These measurements were taken on six roots in each of the four principal calcium chlorid solutions. The averages are given in the following tables, and graphs based upon them are plotted and shown together with the graphs of maximum and average root-hair elongation already reported. The new features here included are: length of the zone of aquatic root hairs in millimeters (z); length of the interzone in millimeters (i); length of the hairless root tip in millimeters (t); length of the entire tip, the sum of the three preceding factors (z, i, t); maximum length of aquatic root hairs, in microns (m); average diameter of aquatic root hairs, in microns (d); and

approximate average distance between adjacent root hairs, in microns (*s*). In addition there are included the graphs of the most rapidly growing root hair (*mr*), and the average rate of growth of root hairs (*ar*).

TABLE 14. Responses of the Root to 0.008 M CaCl_2

pH.....	3.9	4.9	5.9	6.9	7.9	8.9	9.9	10.9	11.4
<i>z</i>	0.0	0.46	2.66	2.33	2.58	3.25	2.42	1.92	0.66
<i>i</i>	0.0	0.0	0.13	0.0	0.0	0.13	0.25	0.38	1.00
<i>t</i>	1.5	1.83	2.33	2.25	2.83	2.58	2.08	2.08	2.08
<i>z, i, t</i>	1.5	2.29	5.12	4.58	5.41	5.98	4.75	4.38	3.74
<i>m</i>		133	466	742	633	683	573	322	98.3
<i>d</i>		21	15	13	13	15	15	18	25
<i>s</i>		50.5	63.3	63.3	71.7	70	46.6	34	

A comparison of the graphs (text fig. 12) for this concentration (0.008 M CaCl_2) leads to the conclusion that the response of the root as shown by maximum length of root hairs is identical with that for rate of elongation of root hairs, except that the graph is shifted one pH unit toward the acid side, the median minimum being at 7.9 instead of 8.9. This difference is doubtless due to a short latent period, that is, a delay in new



TEXT FIG. 12. Responses of roots and root hairs to various hydrogen-ion concentrations of 0.008 M CaCl_2 solutions. *mr*, maximum root-hair elongation; *ar*, average root-hair elongation; *m*, maximum length of aquatic root hairs; *z*, zone of aquatic root hairs; *i*, interzone length; *t*, hairless tip length; *d*, diameter of root hairs; *s*, spacing of root hairs. *z*, *i*, and *t* are given in millimeters; *r*, *d*, *m*, and *s* in microns.

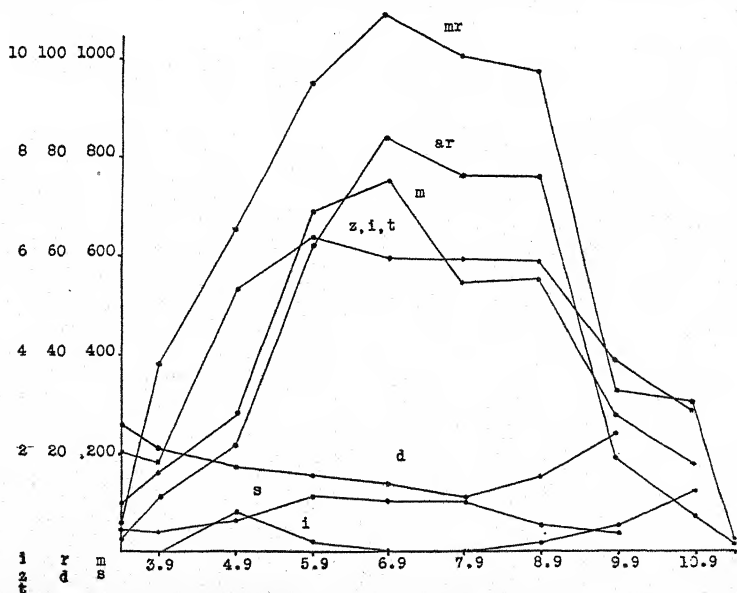
root hairs starting after immersion in the solution. Graphs for *z*, *t*, and *z, i, t* are also bimodal, with a median minimum at 6.9. The diameter of

the root hair and the interzone are at a minimum and the spacing of the hairs at a maximum at 6.9 to 7.9. The maximum length is at a maximum at 6.9 and the rate of elongation at 7.9. These graphs all agree in indicating that the region of 7 to 8 is highly critical for this concentration of the salt. The interzone becomes greater with increased alkalinity beyond the neutral point. The diameter is greatest and the spacing at a minimum in very acid and very alkaline solutions. The maximum root elongation occurs in an alkaline solution, 8.9, whereas the acid optimum for root-hair elongation is greater, namely at 7.9.

TABLE 15. *Responses of the Root to 0.020 M CaCl₂*

pH.....	3.4	3.9	4.9	5.9	6.9	7.9	8.9	9.9	10.9
<i>z</i>	0.29	0.40	2.80	3.42	3.42	3.33	3.00	1.58	0.83
<i>i</i>			0.67	0.17	0.03	0.0	0.06	0.50	1.25
<i>t</i>	1.75	1.40	2.10	3.00	2.50	2.66	2.83	2.00	1.33
<i>z, i, t</i>	2.04	1.80	5.57	6.59	5.95	5.99	5.89	4.08	3.41
<i>m</i>	92	150	270	692	766	550	558	242	180
<i>d</i>	30	20	17	15	13	10	15	24	18
<i>s</i>	43	40	58	106	100	100	45	38	

The graphs (text fig. 13) based upon the data in table 15 and table 9 show an even greater correspondence than in the case of those based upon readings of roots in 0.008 *M* solutions. The graph for average rate of elongation and for maximum length correspond almost exactly, with a



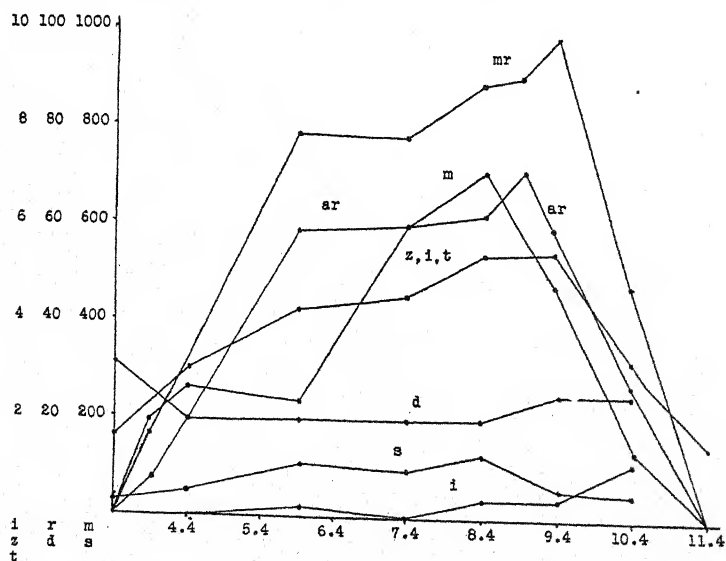
TEXT FIG. 13. Responses of roots and root hairs to various hydrogen-ion concentrations of 0.020 *M* CaCl₂ solutions.

median minimum at 7.9. This is also the median minimum for root length, for diameter of root hairs, and for interzone. The median minimum for spacing is at 6.9, to which the median minimum for interzone also extends. The acid optimum is greater than the alkaline optimum in all curves, except the interzone. All graphs are bimodal. As in 0.008 the graph for length of root tip is shifted toward the acid side as compared with that for root-hair elongation. For all features in which length of root or root hair are involved 8.9 is the alkaline optimum, except in the interzone. With this exception, also, and that for diameter, the acid optimum of all curves is at either 5.9 or 6.9. It may thus be concluded that in all seven of these graphs 7.9 is a critical pH value, in five of them 6.9 is critical, and in four of them 8.9 is critical. Inasmuch as these are the three critical hydrogen-ion concentrations indicated in both graphs based upon root-hair elongation, it appears that the latter is an excellent index of root behavior.

TABLE 16. *Responses of the Root to 0.028 M CaCl₂*

pH.....	3.4	3.9	4.4	5.9	7.4	8.4	9.4	10.4
z.....	0.09	0.58	1.37	1.66	2.33	2.58	2.83	0.83
i.....	0.0	0.0	0.0	0.13	0.0	0.31	0.33	1.33
t.....	1.58	1.66	1.58	2.58	2.33	2.57	2.42	2.33
z, i, t....	1.67	2.24	2.95	4.37	4.66	5.46	5.58	3.29
m.....	56	200	62	242	600	717	475	148
d.....	30	25	220	20	20	20	25	25
s.....	30		50	100	93	125	57	37

A comparison of the graphs (text fig. 14) based upon table 16 with those (text fig. 13) based upon table 15 reveals that whereas in the latter all but

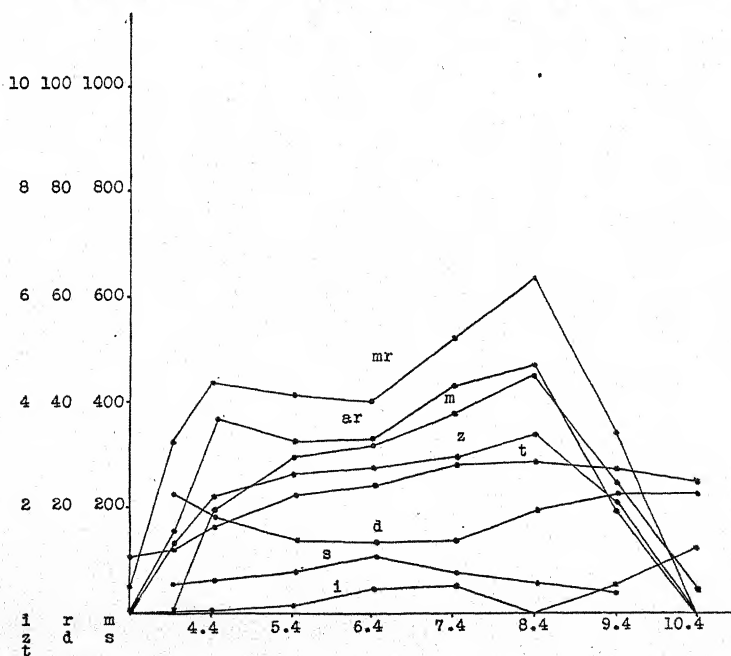


TEXT FIG. 14. Responses of roots and root hairs to various hydrogen-ion concentrations of 0.028 M CaCl₂ solutions.

one of the graphs had the greater optimum on the acid side of the median minimum, all of the graphs of 0.028 solutions have the greater optimum on the alkaline side. For all of these latter graphs except for interzone it falls at 8.4 or 9.4 or between these two pH values. For six of the seven graphs the median minimum is at 7.4. The exception is the maximum length, in which it is at 5.9. As in the case of 0.008 solutions the graph for maximum length of root hair is shifted toward the acid side with respect to that for rate of root-hair elongation, indicating a latent period after immersion. pH 5.9 is the acid optimum in five of the seven graphs, maximum length and diameter proving exceptions. It thus appears that on the basis of all of the graphs constructed upon data from roots and root hairs in 0.028 calcium chlorid solutions, the pH values of 5.9, 7.4, and 8.9 are the critical ones. These are also the three values which are indicated as critical by the graphs based upon root-hair elongation.

TABLE 17. *Responses of the Root to 0.060 M CaCl₂*

pH.....	3.4	3.9	4.4	5.4	6.4	7.4	8.4	9.4	10.4
<i>z</i>	0.0	1.58	2.37	2.83	2.92	3.00	3.58	2.25	0.63
<i>i</i>	0.0	0.05	0.10	0.16	0.54	0.56	0.0	0.56	1.31
<i>t</i>	1.20	1.37	1.75	2.42	2.59	3.16	3.08	2.83	2.66
<i>z, i, t</i>	1.20	3.00	4.22	5.41	6.05	6.72	6.66	5.64	4.60
<i>m</i>	0.0	21	210	317	337	375	466	268	45
<i>d</i>		23	20	15	15	15	22	23	24
<i>s</i>		58	63	83	112	85	63	40	



TEXT FIG. 15. Responses of roots and root hairs to various hydrogen-ion concentrations of 0.060 M CaCl₂ solutions.

As shown by the graphs (text fig. 15) based upon the data in table 17, all of the eight features studied give bimodal curves, with the exception of spacing, for 0.060 *M* calcium chlorid solutions. In all of these seven cases the alkaline optimum is the greater. In five of the graphs the alkaline optimum is at 8.4, while for the other two it is identical with the alkaline limit. In the case of the interzone the median minimum is at 8.4. The median minimum of six of the curves is at 6.4, which is also the pH value of the optimum for spacing, a monomodal curve. The acid limit for three curves is at 4.4, for two other curves it is at 5.4, for diameter it is identical with the acid limit, and for interzone it is at 7.4. Had data been secured at 4.9, it is probable that five curves would have shown that as the acid optimum. It must be concluded then that for 0.060 *M* CaCl_2 , the three critical hydrogen-ion concentrations are represented by pH values of 4.9, 6.4, and 8.4. These are the ones which are also most strongly indicated by the graphs based upon root-hair elongation.

TABLE 18. *Responses of the Root to $\text{Ca}(\text{OH})_2$*

pH.....	6.9	7.9	8.4	8.9	9.9
<i>z</i>	0.0	2.00	1.33	2.00	1.00
<i>i</i>	0.0	0.0	0.75	0.0	0.50
<i>t</i>	2.00	2.00	1.00	2.00	1.16
<i>z, i, t</i>	2.00	4.00	3.08	4.00	2.66
<i>m</i>	0.0	633	166	566	366
<i>d</i>	0.0	15	17	13	15
<i>s</i>		50	50	63	37

The data for these features in calcium hydroxid, presented in table 18, exhibits a bimodal curve for all factors except spacing, which is in harmony with the results for solutions of calcium chlorid and for root-hair elongation in calcium hydroxid. The median minimum for four of the six factors shown in this table with bimodal curves is at pH 8.4; for the other two and for root-hair elongation it is at 8.9. As with solutions of calcium hydroxid, then, it would seem that root-hair elongation is an excellent index of root development, as affected by changes in hydrogen-ion concentration.

DISCUSSION

The seven factors of root and root-hair development upon which data is given in the above tables may here be discussed severally. The spacing of the root hairs on the root is apparently a function of the amount of elongation of the superficial cells of the root in a direction parallel to the axis of the root. In three of the five solutions studied it presents a monomodal curve with the optimum at the median minimum of the graph for root-hair elongation. In the other two solutions (0.020 and 0.028) it is slightly bimodal with the slightly depressed median minimum at the median minimum of the growth curve. This is evidently another expression of

the correlation between elongation of the epidermal cells in the two directions, respectively, as was noted above in connection with a discussion of the lateral movement of the root hairs during the first hour or two of their development when grown in air. It is, however, to be noted that spacing is controlled not by the reaction of the epidermal cell alone to the medium, but of the entire root. The correspondence of the critical point in the curve for spacing with the median minimum for root-hair elongation therefore indicates that the response of the entire root to the solution is much the same as that of the root hair, though the latter gives more consistent and decisive results.

The diameter of the root hair gives in response to variation in hydrogen-ion concentration a bimodal graph, with the two modes at the acid and alkaline limits, respectively; and a middle portion which is flat, except in one (0.020) of the four solutions, in which it is slightly more depressed at one point. It perhaps might better be regarded as an inverted monomodal curve, that is, the root hair is broadened in solutions of high or low hydrogen-ion concentrations. The middle of the flat portion of the curve is very close to the median minimum of root-hair elongation. The diameter of the root hair may be interpreted as resulting from the extent of the growing area of the tip. Further evidence that this is the case will be presented in the following paper (VI). From the data here it seems that the effect of very high or very low hydrogen-ion concentrations is to increase the growing area. Except in 0.008, where the median minimum and the maximum growth pH values are very close together, the minimum diameter does not coincide with the maximum rate of elongation of root hairs. The two curves cannot therefore be said to be the reciprocals of each other. On the other hand, the occurrence of a minimum diameter at the pH value of the median minimum of elongation serves to emphasize the importance of this critical hydrogen-ion concentration.

The graphs for maximum length of aquatic root hairs are of the same form in each concentration as is the average rate of elongation curve; and in two concentrations (0.020 and 0.060) they have the same five critical pH values, that is, limits, optima, and median minimum. In the other two concentrations the optima and median minimum are shifted toward the acid side, but the limits are the same. This has been interpreted above as the result of a possibly greater delay in the emergence of root hairs in some solutions than in others. On account of this variation of latent period with molar concentration, as shown by text figure 11, and with hydrogen-ion concentration, as shown by the curve for interzone, the rate of elongation of root hairs during an interval of three hours beginning 13 hours after immersion is to be considered as a more precise expression of root-hair growth than is maximum length of aquatic root hairs.

In all four molar concentrations the interzone reaches a maximum size at the alkaline limit. It has a median minimum of zero, which extends

over a range of at least one pH unit in two concentrations (0.008 and 0.020). It has an acid optimum also, and below that falls to zero again at the acid limit. The median minimum of the interzone curve is at the same pH value as the median minimum of the root-hair elongation curve in two molar concentrations (0.020 and 0.028). It is at the pH value of the maximum rate of root-hair elongation in three concentrations (0.008, 0.020, and 0.060). This indicates that the initiation of the formation of root hairs is not identical with the rate of their elongation after they start to form. Apparently they are produced best in very acid or neutral solutions, and their production is longest delayed in alkaline solutions. The graphs of the elongation of the entire root tip show that the greater interzone in alkaline solutions is not the result of more rapid root elongation, but must be due to inhibition of root-hair production, although the root hairs may grow quite rapidly in these alkaline solutions, once they are formed.

In all cases except 0.008 the graphs for elongation of the entire root tip follow very closely that for root-hair elongation, having the same optima and median minimum, except in 0.020 where the acid optimum and the median minimum are slightly more acid. In 0.008 the curve is bimodal, but it is higher on the alkaline side and the median minimum is distinctly more acid. This lack of entire consistency in results further serves to emphasize the more definite and distinctive response which the root hair gives to variation in hydrogen-ion and molar concentration than is secured from readings of the elongation of the entire root.

Reference has already been made to a number of investigations of the effects of ionic concentrations upon root elongation. Conspicuous among these is the recent work of Mevius (160) and of the Treleases (94, 95). Other papers are by Maquenne and Demoussy (70) and Robbins and Maneval (86). All of these and many others make valuable contributions to our knowledge of the response of roots to ionic conditions in the medium, but they may now be profitably supplemented by a study of root hairs.

SUMMARY V

50. Data are presented as to the length of the root tip, the zone of aquatic root hairs, the hairless tip, and the interzone between the aquatic and amphibious root hairs for the time of 12 hours after immersion in calcium hydroxid and of 0.008, 0.020, 0.028, and 0.060 *M* calcium chlorid in the different hydrogen-ion concentrations. Similar data are given for maximum length of aquatic root hairs, and for their diameter and spacing.

51. It is found that there is a correlation between the rate of elongation of the root hairs and these other features, especially with respect to the median minimum and the greater maximum in each case. Spacing and diameter of the hairs is at a maximum and minimum, respectively, at the pH of the median minimum for root-hair elongation. The interzone is also usually at a minimum at this hydrogen-ion concentration. The

maximum length of root hairs and the lengths of the zone of root hairs and of the entire tip give curves which correspond quite closely to that of growth rate of the hairs.

52. There is a latent period after immersion during which the root elongates but produces no root hairs. The amphibious root hairs do not elongate during this period either, but renew elongation thereafter. This period may last for as many as three hours, and is at a minimum in solutions of median molar or hydrogen-ion concentration, and at a maximum in alkaline solutions.

53. It is concluded that the entire root responds to changes in hydrogen-ion concentration in much the same way as do the root hairs; but, due to the complexity of its structure and hence of its responses, the results are not as clear cut and as consistent as are those of root-hair elongation.

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PARTITION WALL FORMATION IN THE POLLEN MOTHER CELLS OF *ZEA MAYS*

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Until the publication by Farr (11-14) of a series of papers beginning in 1916, it was commonly thought that partition wall formation in pollen mother cells occurred by the cell-plate method. His investigations showed that in a number of dicotyledons and in at least one monocotyledon (*Sisyrinchium*) the partition walls are formed by a process of furrowing. Farr's findings have been substantiated by Lubimenko and Maige (25) in the Nymphaeaceae, Castetter (7, 8) in *Melilotus* and *Cucurbita*, Gates (18) in *Lathraea*, and Wanda K. Farr (15) in *Cobaea*. In some of these cases a cell plate appears in the heterotypic and homeotypic divisions and then disappears without playing any important rôle in cell division.

Previous to the publication of Farr's work, many investigators had reported partition wall formation by cell plates in the pollen mother cells of monocotyledons, among them being Juel (19) working with *Hemerocallis*; Miyake (26) with *Galtonia*; Farmer (10), Mottier (27), and Schaffner (30) with *Lilium*; Strasburger (32) with *Anthericum*; and Wiegand (35) with *Potamogeton* and *Convallaria*. Numerous instances of division by cell plates are on record, as is shown by Farr, but in none of these cases does the author describe or figure the process in detail. In many cases which have been reported the investigators have been particularly interested in nuclear phenomena, and have studied partition wall formation only incidentally. Several plants in which division had been thought to occur by the cell plate method have been reinvestigated by Farr, who found that division occurred by a process of furrowing.

The literature dealing with partition wall formation in pollen mother cells by the cell plate method is fragmentary and inconclusive. It is thought desirable, therefore, to present the situation found in *Zea Mays* in considerable detail.

MATERIAL AND METHODS

This study was made on Stowell's Evergreen sweet corn which had been selfed once and was relatively pure for its varietal characters. The results were verified with selfed material of Black Mexican and Lancaster. Studies were made on plants grown in both field and greenhouse, and a careful observation made to determine whether any differences occurred as a result of plants being grown under different conditions.

The best sections were obtained by removing the young anthers from

the spikelets, killing them in Bouin's fluid, and staining with safranin and gentian violet. Fresh material was used in making the microchemical tests.

OBSERVATIONS

While the anther is very small the protoplasts of the pollen mother cells are surrounded by a clear homogeneous substance, which has been identified as callose by its reaction to resorcin blue (lacmoid), gentian violet, and aniline blue. It constitutes the mother-cell wall and is thicker in regions where the protoplasts are not in close contact. Such thickenings are most prominent in the central region of the locule (Pl. II, figs. 1, 2) and may be seen on the mother cell at any later stage of development.

As the anther enlarges the sporocytes separate from each other and become arranged around the periphery of the locule leaving an unoccupied central region (figs. 3, 15).

THE HETEROTYPIC DIVISION

The plane of the heterotypic division of the pollen mother cell is cross-wise of the locule (fig. 3). During the anaphase, as seen in figure 4, the spindle fibers are more numerous in the regions between the equatorial zone and the poles than in the equatorial zone itself. This is due to the fact that only those fibers which have no connection with chromosomes cross the equatorial zone. The converging points of the spindle fibers are usually near the periphery of the cell.

As the chromosomes come nearer the poles, the points of convergence of the spindle fibers move toward the equator (fig. 5). The spindle fibers shorten and thicken, gradually withdrawing from the poles, leaving a rather clear region in the vicinity of the nucleus from which they have withdrawn (fig. 6). Stages are sometimes found in which the thickenings of the fibers are above and below, rather than directly at, the equator, resulting in the appearance of a light area between the regions of the thickenings (fig. 6). Slightly earlier stages show the thickened portions nearer the daughter nuclei than is shown in this figure. The spindle appears to be of about the same thickness in this light area as in the equatorial zone shown in figure 4. This, together with the fact that the fibers gradually shorten (figs. 5-10), affords good evidence that the contraction of the spindle fibers begins at their polar extremities. Timberlake (34) states that in the somatic cells of the onion a carbohydrate region is formed across the equator of the cell giving the equatorial region a lighter color; but in the pollen mother cells of the larch he finds the same condition as is described here, and considers it as evidence that kinoplasmic activity preparatory to the formation of the cell plate begins in the region of the nucleus. Radiating fibers, which have been described by other investigators, have been occasionally observed by the writer in the telophase of the heterotypic division in the pollen mother cells of *Zea Mays*. The fibers of the central

spindle now become of a uniform thickness throughout their entire length (fig. 7). At this stage the chromosomes are in close contact with each other, forming a dense mass.

Definite thickenings are then formed on the spindle fibers at the equator of the cell (Pl. II, fig. 8 *b*) in a manner similar to that figured by Allen (2) in *Lilium*, Suessenguth (33) in *Panicum*, and Stenar (31) in *Haemanthus*, *Bomarea*, and *Hypoxis*. The thickenings of the fibers increase in size until they come in contact with each other and fuse together, forming the cell plate. All of the thickenings of the original spindle do not fuse simultaneously, however, and as a result they form what first appears as a broken line along the equator (fig. 8 *a*), then as a continuous line—the cell plate (fig. 9). All stages have been found between the formation of thickenings on the spindle fibers and the stage shown in figure 9.

Spindle fibers continue to appear just beyond the periphery of the plate, increasing the diameter of the spindle (figs. 9–13). Timberlake (34) is inclined to think that in the pollen mother cells of the larch new fibers are not added, but that the growth of the cell plate occurs as a result of changes in the existing fibers. In the onion, however, he thinks that the conditions are such as to necessitate the formation of new fibers. The present writer is of the opinion that in *Zea Mays* new fibers are formed in a manner similar to that found by Timberlake in the onion. The fibers in the region of the central spindle disappear long before the plate has reached the periphery of the cell. During the time of the formation of peripheral fibers the cell plate has never been seen to extend to the periphery of the spindle, indicating that the formation of peripheral fibers must precede the centrifugal development of the cell plate. Bailey (3, 4) has shown that in the tangential division of elongated cambium cells the spindle fibers have disappeared from the wall in the middle portion of the cell while cell-plate formation is still in progress near the periphery.

The older portion of the cell plate now appears thicker and stains lighter with gentian violet than when first formed. The spindle fibers along this portion of the plate disappear rapidly (fig. 9), and when they have practically disappeared the plate begins to split (fig. 10). The splitting does not necessarily begin in the middle of the cell, but usually somewhere in the region which was formed from the original central spindle.

After the cell plate has begun to split, a rather homogeneous substance can be recognized between its two halves (figs. 10–12). At a later stage it is observed that the region along the middle of the new cell wall stains darker than the rest of the wall (fig. 13). This dark-staining region occupies the exact position of the middle lamella, but upon microchemical examination it proves to be of a different composition. When fresh material is treated with ruthenium red or methylene blue, this region fails to give the test for pectic materials, as is normally the case with the middle lamella. But when treated with lacmoid or aniline blue the entire new wall gives a

reaction for callose, the middle region staining a deeper blue than the rest of the wall. When stained with resorcin blue this middle region is fairly distinct.

Although this structure is a layer of dense callose, from the standpoint of its origin it is the primary wall. The same structure has been shown in the pollen mother cells of *Ipomoea* and *Oenothera* by Beer (5, 6), who states that it gives the reaction for callose. He designates it as the middle lamella and believes that it represents the first lamellae deposited after the completion of cell division. A structure similar to this has been reported by Castetter (8) in *Cucurbita*. In the above cases the partition wall is structurally the same as is found here, and this middle lamella also represents the region where the secretions from the two daughter protoplasts are in contact. But the method of wall formation in *Cucurbita*, and apparently in *Ipomoea* and *Oenothera* also, is entirely different, the walls in those cases being formed by furrowing. Laminations are present in the callose walls of *Zea* as was also reported in *Cucurbita*. Strasburger (32) showed a structure in *Lilium* which resembles this primary wall rather closely, and Lubimenko and Maige (25) have figured a similar structure in *Nuphar luteum*. It seems probable that these structures figured by Strasburger and Lubimenko and Maige are identical with that observed in *Zea*.

About the time the cell plate is definitely formed, the chromatin of each daughter nucleus loosens up and a nuclear membrane is formed around it. Numerous dark-staining bodies have been observed in the cytoplasm, and are seen to persist until near the close of the homoeotypic division. Their significance has not been determined, although they have been described by Kuwada (20) as extra-nuclear nucleoli. They react as nucleolar material to Heidenhain's haematoxylin and iron acetocarmine, and with the latter stain they are easily distinguishable from chromosomes. These bodies occur throughout the cytoplasm, sometimes very close to the daughter nuclei or in the converging points of the spindle fibers.

A short time before the partition wall breaks through the periphery of the mother protoplast, one or more irregularly shaped nucleoli are observed. This is the earliest stage in which the writer has observed the nucleolus.

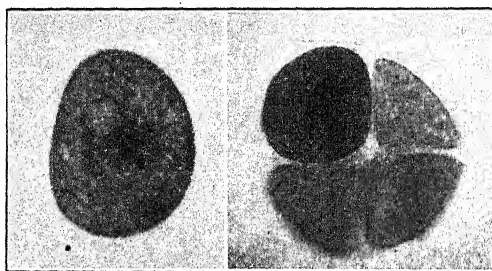
By the time the wall has broken through the periphery of the mother protoplast, the peripheral fibers have almost entirely disappeared. Only a trace of them can be seen in the angles formed by the new wall and the wall of the mother cell (fig. 13). Special precaution was taken to use well fixed material for stages represented by figures 10 to 13, as in these stages plasmolysis causes the cell membrane to draw away from the new partition wall, allowing the wall to become wrinkled and making the recognition of its parts quite difficult. The chromatin of the nucleus now tends to collect along the nuclear membrane.

The daughter cells are now completely formed, the partition wall having joined with the wall of the mother cell, and the angles having rounded off

(Pl. III, fig. 14). Each half of the split cell plate has become the adjacent part of the cell membrane of a daughter protoplast. At about this stage the daughter nuclei attain their maximum size, the chromosomes become more definite, and the nucleolus large and conspicuous. When stained with safranin and gentian violet, distinct vacuoles can be seen in the nucleoli.

THE HOMOEOTYPIC DIVISION

The plane of the homoeotypic division is radial-longitudinal with the locule (Pl. III, fig. 15). The homoeotypic and heterotypic divisions are essentially alike, and for that reason the homoeotypic division is reported here in less detail. The fibers of the homoeotypic spindle contract and thicken at the middle and these thickenings fuse, forming the cell plate (fig. 17). In this figure the dark masses of chromosomes are loosening up to form what is ultimately seen in figure 19. From the cell plate shown in figure 17, a new partition wall develops just as in the heterotypic division. This wall grows until it breaks through the periphery of the daughter protoplasts, making a tetrad with the bilateral arrangement of microspores (fig. 19). When using living material it is not difficult to break the wall of the tetrad and cause the microspores to drift out of the callose wall. At a later stage when the microspores are slightly older than those shown in



1

2

TEXT FIG. 1. A young microspore showing large vacuole.

TEXT FIG. 2. Tetrad of microspores with three of the members aborting.

figure 19, the callose wall disintegrates and releases them. The primary wall is observed to be the last part of the wall to disintegrate. The microspores when fully formed are smooth, almost spherical bodies, containing from one to several large vacuoles (text fig. 1).

ABNORMAL CONDITIONS

Figure 20 represents a cell which is really four microspores enclosed within the mother-cell wall. Nuclear division was completed and the grand-daughter nuclei were formed without a partition wall between them. This condition was found in only one plant; this one, however, furnishing an

abundance of such material, but exhibiting no other noticeable abnormal condition. A similar situation has been reported by Kuwada (20) in *Zea*, in which a constriction is formed in the mother-cell wall partially separating the daughter nuclei. But his description does not conform well to this case, in which no constriction of the mother-cell wall has been observed.

Such abnormal cell divisions sometimes prove important in connection with abnormalities in chromosome content and in heredity. Abnormalities of chromosome content do sometimes occur in *Zea Mays* as has been shown by Fisk (16, 17), Kuwada (21, 22), Longley (23), Randolph and McClintock (28), and the writer (29). No relation, however, between abnormal cell division and abnormal chromosome behavior has been observed.

In text figure 2 a tetrad is shown in which three of the members are aborting while the other one is developing normally. Any number of the members of the tetrad may abort. This is of frequent occurrence in material grown in the greenhouse, and is probably a nutritional phenomenon.

DISCUSSION

The origin and nature of the walls in somatic cells have been carefully studied by a number of investigators. Timberlake (34) found that material flows from the ends of the fibers to the equator, and there swellings are formed accompanied by a shortening of the fibers. These swellings fuse, forming the cell plate. The cell plate splits, each half of it becoming the adjacent part of the cell membrane of a daughter cell. A middle lamella is then secreted between the two cell membranes, also a secondary wall on each side of the middle lamella. Allen (1) later showed that this middle lamella was composed of pectic material and was of double nature. Much the same process was reported in the pollen mother cells of *Larix* by Timberlake. Devise (9), however, has come to the conclusion that the cell plate in *Larix* originates from spindle-fiber substance after the first mitosis and then disappears; and that after the second mitosis another cell plate arises out of the homogeneous cytoplasm and takes an active part in cell division.

Lutman (24), working with somatic cells, is of the opinion that the spindle fibers are hollow tubes. He states that the chromosomes pass through the traction fibers to the poles in an amoeboid fashion, and liquid cytoplasm passes through the connecting fibers from the equatorial region to the poles to assist in hydrolization of the chromatin and linin. The cell plate arises as a series of fused vacuoles and is essentially the result of a drying-out process.

The figures in the present paper, however, show that the fibers contract from the daughter nuclei toward the newly formed cell plate. They could not, therefore, be instrumental in carrying material to the daughter nuclei, except in stages earlier than cell-plate formation. The process reported here is similar to that described in somatic cells by Timberlake and by Allen, but there are two significant differences: a middle lamella composed of

callose, instead of pectic materials, is formed between the split halves of the cell plate; and secondary walls are then secreted which are identical in position with the secondary walls described by Timberlake, but different in that they are composed of callose. However, in such cases as have already been mentioned where the partition wall (both primary and secondary) is composed of callose, the wall has been formed by furrowing.

SUMMARY

Partition walls in the pollen mother cells of *Zea Mays* are formed by the cell-plate method and, contrary to the usual situation found in walls formed in this way, both primary and secondary walls are composed of callose—a combination of features not previously reported in detail.

The writer wishes to express his thanks to Dr. E. F. Castetter, who suggested this problem and has given advice and criticism during the course of the entire work; and also to Dr. J. N. Martin for his interest and valuable suggestions.

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EXPLANATION OF PLATES

The approximate magnification of each figure is given.

PLATE II

FIG. 1. Longitudinal section of young anther lobe showing callose along the axis of the locule. $\times 145$.

FIG. 2. Cross section of same. $\times 145$.

FIG. 3. Longitudinal section of anther lobe at time of heterotypic division, showing plane of division. $\times 145$.

FIGS. 4-14. Heterotypic division.

FIG. 4. Anaphase. Spindle fibers converging near periphery of cell. $\times 1300$.

FIG. 5. Points of convergence of spindle fibers have moved inward. $\times 1300$.

FIG. 6. Spindle fibers contracting. Light area across equator of spindle. $\times 1300$.

FIG. 7. Spindle fibers continuing to contract. They are of rather uniform thickness throughout their length. $\times 1300$.

FIG. 8 *a*. Spindle continuing to contract. Swellings are seen on fibers at the equator of the spindle. $\times 1300$.

FIG. 8 *b*. Spindle fibers showing distinct enlargements at the middle. $\times 1500$.

FIG. 9. Cell plate definitely formed. Fibers of equatorial region becoming very short. Nuclear membrane present. Peripheral fibers forming. $\times 1300$.

FIG. 10. Fibers of equatorial region rapidly disappearing. Cell plate splitting. Homogeneous substance appearing between the split halves. $\times 1300$.

FIG. 11. Fibers of original spindle have completely disappeared. Homogeneous substance continuing to form between the two halves of the cell plate. $\times 1000$.

FIG. 12. Later stage. $\times 1300$.

FIG. 13. Middle lamella visible. Partition wall breaking through surface of mother cell. Nucleoli present. $\times 1300$.

PLATE III

FIG. 14. Daughter protoplasts have rounded off. $\times 1100$.

FIGS. 15-19. Homoeotypic division.

FIG. 15. Cross section of locule at time of homoeotypic division showing plane of division. $\times 145$.

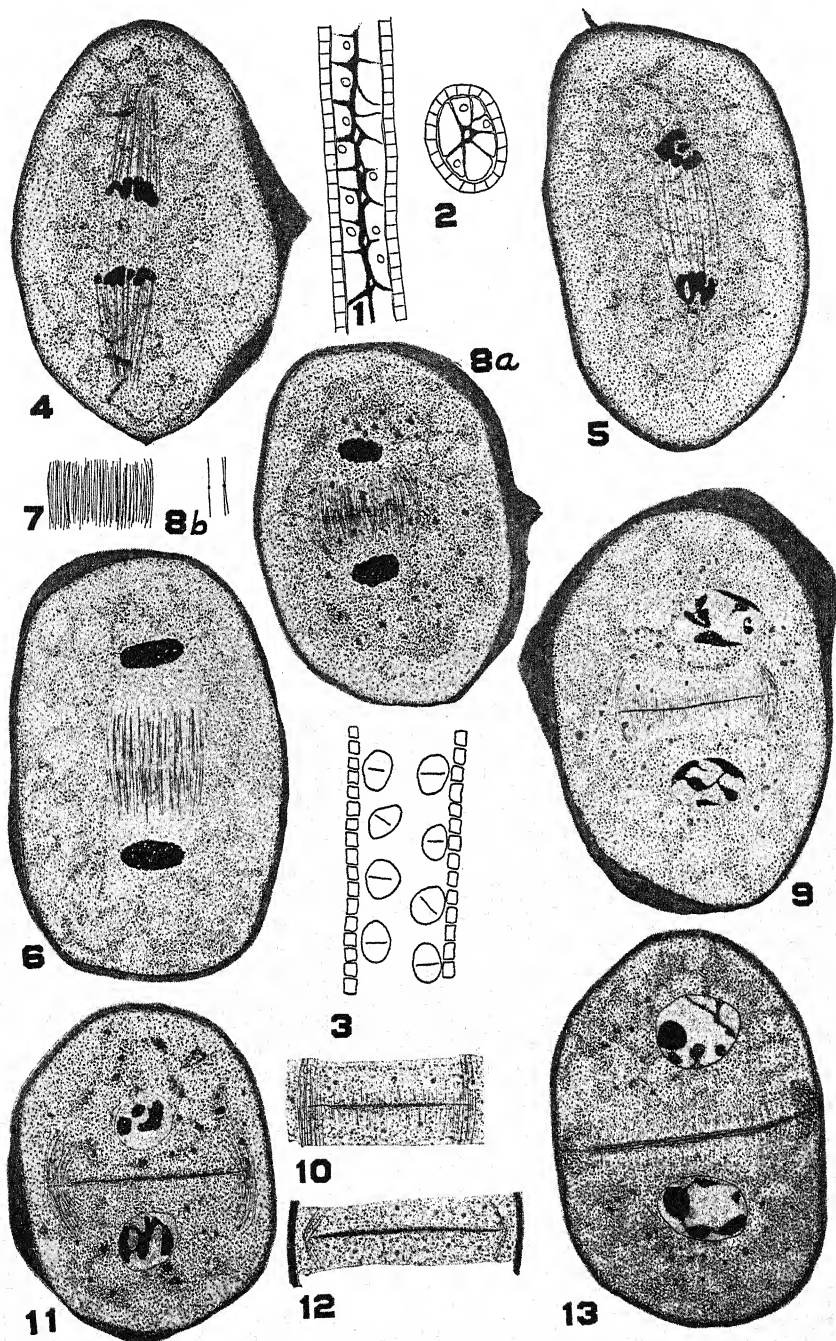
FIG. 16. Daughter cells have elongated. Spindles have thickened along the equator. $\times 1300$.

FIG. 17. Cell plate present. $\times 1300$.

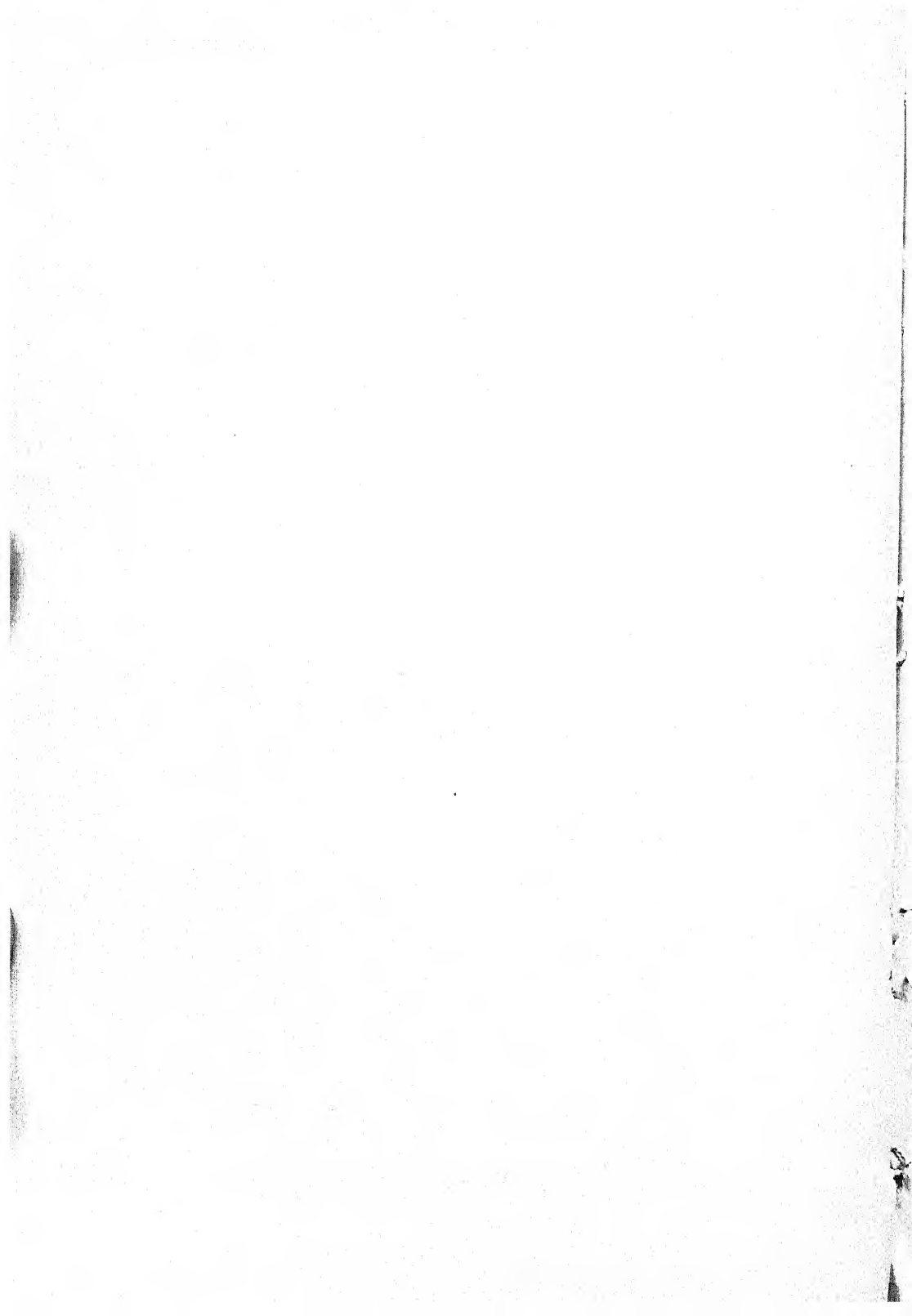
FIG. 18. Granddaughter cells almost completely formed. $\times 1300$.

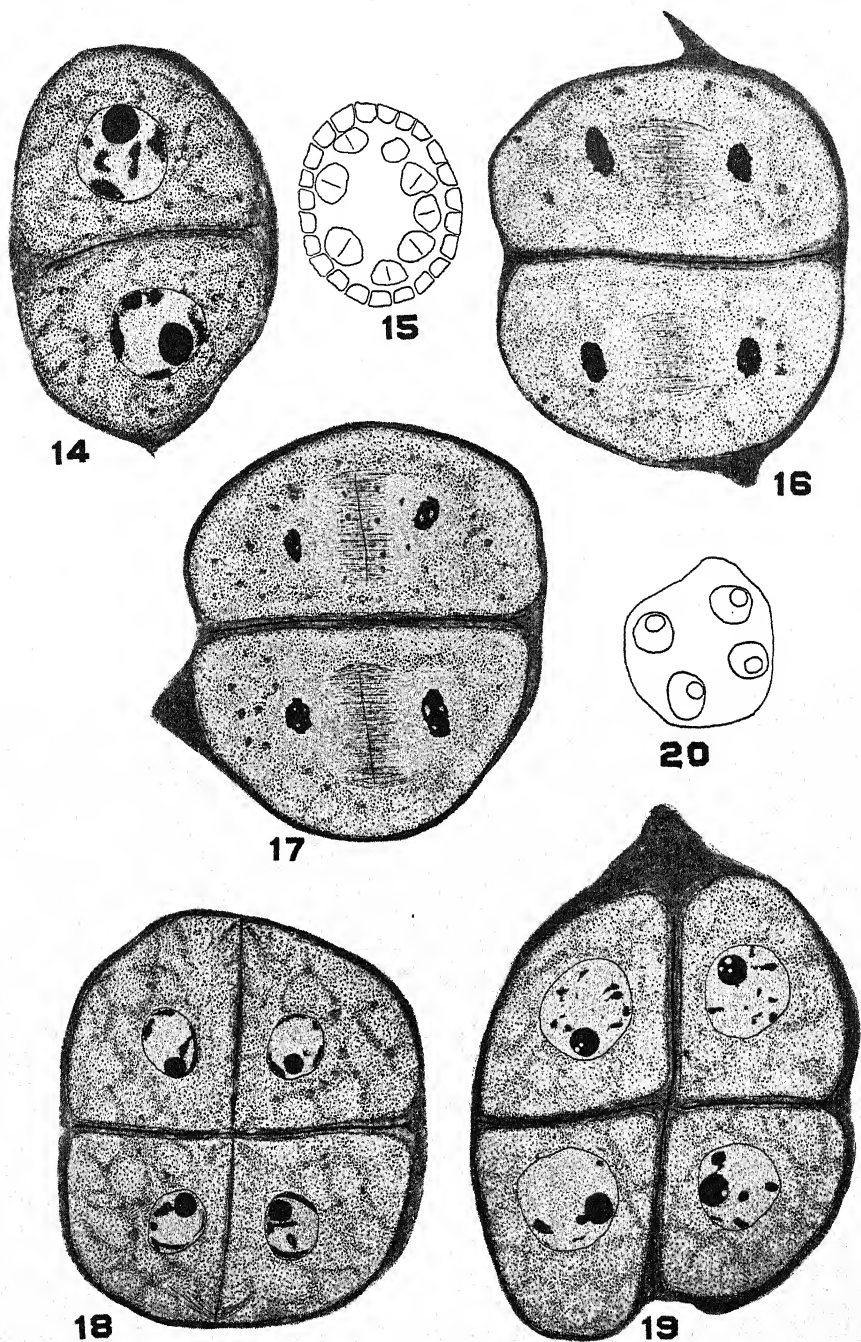
FIG. 19. Tetrad completely formed. Vacuoles visible in the nucleoli. $\times 1300$.

FIG. 20. Abnormal condition. Divisions have been completed without the formation of partition walls. $\times 440$.



REEVES: PARTITION WALL FORMATION





REEVES: PARTITION WALL FORMATION

FEEDING HABITS OF THE SWARM CELLS OF THE MYXOMYCETE *Dictydiaethalium plumbeum*¹

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INTRODUCTION

The swarm cells of Myxomycetes formerly were believed to take in food only in solution, and in 1887 DeBary (1) expressed this general opinion. Two years later, however, Lister (3) definitely showed that the swarm cells regularly engulfed and digested bacteria. He also showed that they sometimes ingested unicellular algae and particles of inorganic matter but later discharged these, apparently unchanged. Since that time, many authors have observed that swarm cells feed upon bacteria, but a survey of the literature fails to disclose any report of fungous spores serving as food. Most Myxomycetes, in their later plasmodial stage, engulf and digest microcysts, spores, bits of hyphae, Protozoa, and other organic material, and the writer has observed that certain species, in culture, may creep over bits of bark and wood, completely removing all traces of fungous and other growths that were present and leaving only an excreted brown slime. Similarly, certain free-living amoebae which resemble swarm cells and myxamoebae in many respects, may feed upon many small organisms, including spores and bacteria. Inasmuch as plasmodia and free-living amoebae show such phytophagous traits, it was of interest to determine whether or not the swarm cells show similar feeding habits, since in their natural habitat on the moist undersides of rotting logs and other substrata they necessarily become closely associated with bacteria, fungous spores, and yeasts. With the purpose, therefore, of finding if the swarm cells do indeed show such feeding traits, detailed investigations were made, the results of which are given in part in this paper.

MATERIALS AND METHODS

Dictydiaethalium plumbeum was chosen among a number of species that were tried since it combined the most desirable qualities for the experiments, namely that the spores germinated well in a short time and the large swarm cells readily passed to a creeping stage, during which feeding was found to take place. The majority of the filamentous fungi whose spores were used to feed the swarm cells were isolated from rotten wood or other substrata normally occupied by Myxomycetes, a few were from stock laboratory cultures of common moulds, while *Penicillium*

¹ Contribution from the Cryptogamic Laboratories of Harvard University, no. 95.

roquesforti and *Penicillium camemberti* were obtained from commercial cultures used in cheese manufacture.

The study of the feeding habits of the myxomycetous swarm cells was carried out chiefly in Van Tieghem cells in commercially distilled water of pH 6.8. The spores of the myxomycete and the fungus were usually sown together, but if the latter were of a type that soon gave rise to mycelium it was necessary to sow the myxomycetous spores first and then, when the swarm cells appeared, to add the spores of the fungus. The reactions of the swarm cells to the spores was recorded by camera lucida drawings and by micro-photographs, the latter being taken with a Leitz "Makam" Mikraufsatzkamera. In taking these, heat-absorbing glass was placed between the object and the source of light, and also a piece of thin white paper while focusing and adjusting, since strong light and heat were found to cause cessation of feeding and rapid encystment of the swarm cells (Pl. V, fig. 1). The cover with the drop was removed from the Van Tieghem cell and placed on a very shallow, ground-glass slide so that the drop just filled the depression, thus preventing that condensation of water on the cover which would necessitate constant change of focus. Since the index of refraction of the swarm cell and that of the water were nearly the same, there was some difficulty at first in securing contrast in the photographs, but by swinging the substage mirror so that the rays reached the object obliquely, this difficulty was overcome.

LIFE HISTORY

The life history of *Dictydiaethalium plumbeum* is typical of the majority of Myxomycetes and takes place in the following manner: a spore in germinating gives rise to a uniflagellated swarm cell which feeds and grows and has two distinct movements, first an active rotating one and later a slower, more or less amoeboid, creeping movement. The swarm cell divides a number of times and eventually the flagellum is withdrawn, after which the movement is entirely amoeboid. Swarm cells in this condition are termed myxamoebae. A myxamoeba also grows and divides and eventually its daughter cells and those of other myxamoebae fuse in pairs. The pairs or zygotes unite, forming a large multinucleate mass of protoplasm, the plasmodium, which feeds and grows and under favorable conditions forms its fructifications, completing the life history. Only the swarm cell stage is considered in this paper.

INGESTION OF FUNGUS SPORES

The swarm cells of *Dictydiaethalium plumbeum* are from ten to twenty microns in length and from five to ten microns in width, hyaline or finely granular, with a single anterior flagellum about twice the length of the body. The nucleus is well marked and is situated at the anterior end of the swarm cell, a little below the base of the flagellum. The swarm cell has two distinct movements as stated above, and during the creeping move-

ment will ingest fungous spores (Pl. V). These are engulfed in essentially the same way as bacteria. This occurs as follows: a tenuous pseudopodium, put out from the posterior part of the body, attaches itself to a spore or bacterium with which it comes in contact, and then retracts, drawing the spore or bacterium towards the body where extensions of protoplasm fold over and enclose it. This ingestion of solid matter by swarm cells, as far as can be ascertained, occurs only through the posterior end of the body and only during the creeping stage.

Spores of some species of fungi are ingested very readily and at times a swarm cell is found to contain as many as four freshly ingested spores, in addition to the remains of older ones (Pl. V, figs. 3, 4). An ingested spore is enclosed in a vacuole and may rest in any part of the cell, with the exception of the anterior region near the nucleus, but is frequently moved to various places in the body during the activities of the organism. Eventually, after a number of hours, the spore becomes smaller and more indistinct and is digested, with the exception of certain portions such as oil globules which are thrown out.² These are brought to the surface of the protoplasm in a vacuole, and for the most part are merely voided in the water by the contractile action of the vacuole but occasionally may be expelled almost explosively, as the vacuole suddenly empties itself. In an old culture there are numbers of these oil globules floating free, sometimes with ragged remnants of the spores attached to them.

The filamentous fungi used in the experiments, as stated, were from three sources. The majority were common moulds isolated from rotten wood and other substrata commonly occupied by Myxomycetes. *Mucor ramannianus* A. Moell., *Acrostalagmus fragrans* Cr., *Trichoderma lignorum* (Tode) Harz, and *Aspergillus niger* Van Tieghem are examples of this type. A number of species of *Penicillium* were also isolated from rotten wood but, as extreme difficulty was experienced in attempting to identify them, cultures of *Penicillium camemberti* Thom and *Penicillium roqueforti* Thom were secured in order to have authentic specimens of this genus. Some of the fungi, for example *Oospora humi* Mazé and *Dematium Chodati* Nech., were from laboratory cultures of long standing and were used to give additional data upon the feeding habits of the swarm cells, even though they probably seldom or never occur in situations where swarm cells might be found.

The fungi used in the experiments may be classified in another manner, depending upon the degree to which their spores are ingested by the swarm cells. In the first of these classes, represented by *Monilia candida* Bon., *Candida breve* Berkhout, *Acrostalagmus fragrans* Cr., *Oospora humi* Mazé, *Dematium Chodati* Nech., and *Mucor ramannianus* A. Moell., the spores are ingested and digested very readily. Swarm cells feeding on these species are often packed with spores, become very large, and divide at a rapid rate.

² That these were globules of oil was demonstrated by staining with Sudan III and with osmic acid.

Division often takes place with the spores still undigested within them, in which case each daughter cell may receive a part of the original number of spores (Pl. IV, figs. 1-6). It may be inferred that spores of filamentous fungi of this type could easily constitute a part of the natural food of the myxomycetous swarm cells, in addition to bacteria and soluble matter.

In the second category fall species that, although apparently attractive to the swarm cells, form spores which are either too large to be taken in, or germinate so quickly that they almost immediately form hyphae too great for the swarm cells to ingest. Species falling in this class are *Monilia sitophila* (Mont.) Sacc., *Chaetomium cochlioides* Palliser, *Clonostachys* sp., and *Hormodendron* sp.

Swarm cells were not found to ingest spherical spores over eight microns in diameter, or about one-fifth of their volume. This ratio was obtained by first carefully measuring a number of swarm cells and afterwards the largest of the spores that they ingested. Plasticine models then were made of swarm cell and spore, and the volume of water displaced by each recorded. The ratio between spore and swarm cell is thus found to be approximately one to five. Zygotes and young plasmodia may take in larger spores than those taken in by the swarm cells but the ratio of one to five is not exceeded. Spores with germ tubes are ingested if the whole mass is not too large (Pl. IV, fig. 8). Swarm cells often take the ends of long germ tubes into their bodies or wrap themselves around a portion of one, but when observed continuously have always been found to become detached later (figs. 9-11).

Trichoderma lignorum (Tode) Harz., *Pullularia nigricans* Berkhout, and *Spicaria* sp. fall into the third category. Their spores are ingested but there is no evidence that they are digested, for in the many cases observed they were sooner or later thrown out, apparently unchanged. A careful examination of their walls showed no sign of corrosion nor any effect of having been within the body of the swarm cell.

In the fourth class should be included *Penicillium* and *Aspergillus*, which under ordinary circumstances are not ingested at all and even seem to repel the swarm cells. In order to determine whether this repellent action is due to the composition of the spores, a large number were alternately moved from liquid air to warm water in hopes of shattering them and seeing the effect of their contents on the swarm cells. The spores did not shatter, but when placed later in a Van Tieghem cell with a few swarm cells, were taken in, in moderate numbers. This experiment seemed to indicate that some substance, possibly a layer of air on the cell wall, rather than the cell content, repelled the swarm cells, so that after this was removed the spores were ingested. In order to obtain more evidence on this point, *Aspergillus* and *Penicillium* spores were soaked in absolute alcohol, allowed to dry, and then sown in the cultures, where they were ingested as before. Spores thus treated, however, even when ingested, were not digested but were later thrown out unchanged.

To determine whether the swarm cells would ingest inert material, carmine particles and finely pulverized silica were fed to them. Both were taken in occasionally but neither was digested. The swarm cells react to the inorganic substances in much the same way that they do to fungous spores of species of the third and fourth classes in which the spores are ingested to some degree, but are not digested, as far as can be ascertained. This similarity of reaction seems to indicate that the ingestion of spores of these species is a mere mechanical process, involving no positive chemotropism in response to diffusing food material.

COMPARISON WITH OTHER ORGANISMS

The order Myxogastrales, of which *Dictydiaethalium plumbeum* is a member, is regarded as the highest of the four orders of the Mycetozoa, whose taxonomic position has been the subject of much discussion. Without entering into this question, one may safely say that the Mycetozoa have affinities with the amoeboid Protozoa, on the one hand, and with the Chytridiales on the other. Because of these affinities, it is of interest to note similarities or diversities in the related forms.

In the Acrasiales, a fairly large order of seven genera and twenty species, Olive (5), who made a most careful study of the group, found that the myxamoebae ingested solid materials such as bacteria and made use of them in their metabolism. He was able to bring the myxamoebae to maturity in pure culture in nutrient decoctions without the ingestion of any solid particles but says "such cultures when absolutely pure (*i.e.*, free from bacteria) did not develop luxuriantly and the fructifications were fewer in number and smaller than usual."

The Labyrinthulales, a small order, are not so well known as the Acrasiales; three of the five species, *Labyrinthula vitellina* Cienk., *L. macrocystis* Cienk., and *L. Cienkowskii* Zopf, all parasites on algae, having been observed only by their authors. A fourth species, *Diplophrys Archeri* Barker, which occurs in fresh water, is not with certainty referred to the order, while *Diplophrys stercorea* Cienk. has not been studied sufficiently to determine whether or not ingestion of solid particles by the swarm cells takes place. However, Zopf's work (6) on *Labyrinthula Cienkowskii*, a species found on *Vaucheria*, gives strong evidence that in the parasitic species, at least, the swarm cells obtain their food entirely by absorption.

The relatively few members of the Plasmodiophorales are intracellular parasites of seed plants, at least during part of their life, and as far as is known absorb all or nearly all of their food in the liquid state at this time. However, during the time that they may exist as free-living organisms it is very probable that they are capable of making use of solid ingesta, and further investigations on this point would be of great interest.

The Chytridiales (4), the one order related to the groups under discussion that is admitted to the plant kingdom without reservation, contains intra-

cellular or intercellular parasites of both plants and animals. Flagellated swarm cells somewhat similar morphologically to those of the Myxogastrales are developed but have a relatively brief swarming period, serve merely for dispersal, and so far as is known do not feed. In the Olpidiaceae and the Woroninaceae such swarm cells come to rest on their host, round off, penetrate the wall, and discharge an amoeboid mass of protoplasm into the host cell. In the cell this protoplast of the parasite grows, either remaining differentiated or apparently mingling with the protoplasm of the host. In the Synchytriaceae a swarm cell loses its flagellum and penetrates its host directly, feeding on the protoplasm of the cell which it has entered. It is not known, however, whether the swarm cells or amoeboid cells of this family or of the Olpidiaceae and Woroninaceae absorb the protoplasm of the host or ingest it.

The three families mentioned above form the sub-order Myxochytridinae. The other sub-order, the Mycochytridinae, although bearing active swarm cells, does not have an amoeboid thallus and therefore is not so closely related to the Myxogastrales and is not considered here.

The amoeboid Protozoa, members of the lowest animal phylum, are either parasitic or free living, and when free living take in and digest other Protozoa, bacteria, and spores. The method of ingestion is the same as that of the myxomycetous swarm cells and myxamoebae, and when certain members of the two groups are in the same culture, they are with difficulty distinguished. In fact, one common amoeboid contaminant of myxomycetous cultures, *Hyalodiscus guttulata* Duj., is often confused with swarm cells and myxamoebae, especially when all are stuffed with spores. Of course, the presence of a flagellum immediately identifies the swarm cells while the peculiar flowing movement and the single pseudopodium of each amoeba differentiate them both from the swarm cells and the myxamoebae. It is the free-living amoeboid Protozoa, therefore, that show the greatest similarity to the swarm cells in feeding habits since not only is the method of ingestion similar but both take in the same sort of food, and in much the same manner.

CONCLUSIONS

It has been shown that swarm cells of *Dictydiaethalium plumbeum*, a species of the Myxogastrales, in addition to obtaining nutriment by absorption from the liquid in which they live, also engulf and digest fungous spores and bacteria. They simulate, therefore, in their feeding habits, the plasmodium which they become at a later stage and differ only in the degree to which this ingestion takes place. Similarly, the swarm cells in their manner of feeding resemble closely related orders, both animal and fungus.

From the above experiments, a number of questions arise concerning the behavior of the swarm cells towards the material which they ingest. One point seems clear; the swarm cells appear to show no positive

chemotropism toward diffusing food material. In other words, if spores which are easily digested are placed in one corner of the culture, there is no migration of swarm cells toward that corner. The swarm cells that come in contact with the spores by chance ingest them readily, while the other swarm cells behave as if there were no spores in the culture. The fact that inorganic matter and certain indigestible spores are occasionally engulfed and soon cast out again would appear to indicate that the swarm cells are unable to differentiate between material which they can use in their metabolism and material which is of no use to them. However, should the swarm cells be incapable of differentiating between digestible and indigestible spores, in cultures where these two types of spores were sown together in approximately equal numbers so that the swarm cells came in contact with one as frequently as with the other, one would expect to find that they would take in both kinds of spores indiscriminately. This is not the case, however, in cultures where the spores of *Monilia candida* and of *Aspergillus niger* or *Penicillium* sp. that have been wet with alcohol are sown together in equal numbers. The favorable spores of *Monilia candida* are taken in in large amounts so that the swarm cells become crowded with spores, while those of *Aspergillus* or *Penicillium* are very seldom ingested. It seems, therefore, that the swarm cells show some power of selection in their ingestion but why inert material and spores that are not digested are taken in at all, the writer is unable to explain.

Fungous spores are not essential to the swarm cells, as plasmodia may easily be grown from spores without any fungi being present. However, it is very apparent that the presence of certain fungous spores, in cultures of *Dictydiaethalium plumbeum* at least, is of a distinct advantage to the swarm cells, for in distilled water with fungous spores which they were able to digest, they were more thrifty, divided more often, were of a larger size, and remained active longer than in distilled water with no fungous spores present. It seems very probable that spores of certain fungi form one of the main articles of food for the swarm cells of *Dictydiaethalium plumbeum*, in addition to bacteria and soluble matter, and that the ingestion of these spores is not an accidental occurrence.

SUMMARY

In this paper the writer records certain observations on the feeding habits of the swarm cells of *Dictydiaethalium plumbeum*, points which in this species had not been critically studied so far as could be ascertained.

The swarm cells have two types of movement, a rotating movement and a more or less amoeboid creeping movement. During the creeping movement they are capable of ingesting solid matter; and in addition to bacteria and soluble material, the spores of certain types of filamentous fungi may serve the swarm cells as food. The largest spores that are taken in are not greater than one-fifth of the volume of the swarm cell and although

myxamoebae and young plasmodia may ingest spores larger than this, the ratio of one to five is not exceeded. The spores of *Monilia candida* Bon., *Candida breve* Berkhout, *Acrostalagmus fragrans* Cr., *Oospora humi* Mazé., *Dematium Chodati* Nech., and *Mucor ramannianus* A. Moell. are very readily ingested and digested by the swarm cells. The spores of *Monilia sitophila* (Mont.) Sacc., *Chaetomium cochlioides* Palliser, *Clonostachys* sp., and *Hormodendron* sp. are not ingested because in the first two species they are too large and in the others they germinate so quickly that they soon form hyphae too great for the swarm cells to take in. The spores of *Trichoderma lignorum* (Tode) Harz., *Pullularia nigricans* Berkhout, and *Spicaria* sp. are ingested but are later thrown out, apparently unchanged. No spores of any of the eight common species of *Aspergillus* and *Penicillium* used are ingested under ordinary conditions, although after alternate freezing and thawing or after being immersed in absolute alcohol they are taken in occasionally but are always later ejected. Inert bodies, such as fine particles of carmine and silica, are taken in but never digested, and are always after a short time thrown out unchanged. Oil globules that may be ingested in a spore containing them are never digested, but are expelled after more or less of the remainder of the spore has been absorbed. After expulsion, the oil globules, being free from the rest of the spore which formerly held them down, rise to the top of the water.

The writer is greatly indebted to Miss Margaret B. Church for authentic cultures of *Penicillium*; to Dr. David H. Linder for help in taking microphotographs, and for aid and criticism to Dr. William H. Weston, Jr., under whose supervision the work was performed at the Laboratories of Cryptogamic Botany of Harvard University.

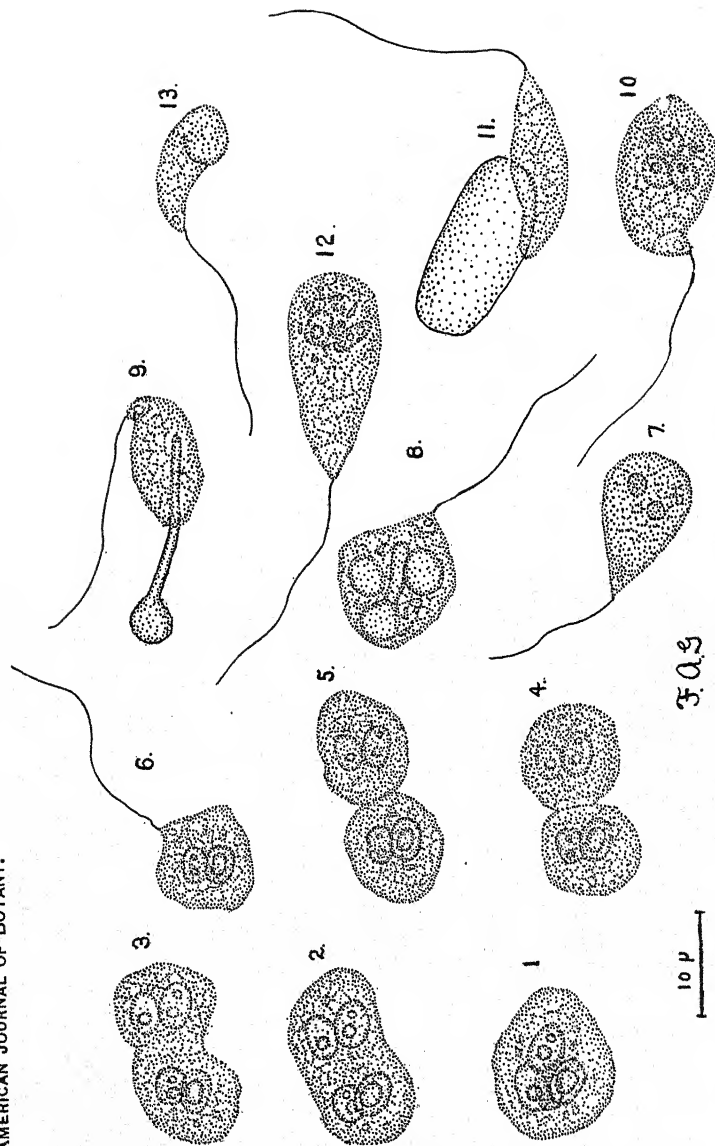
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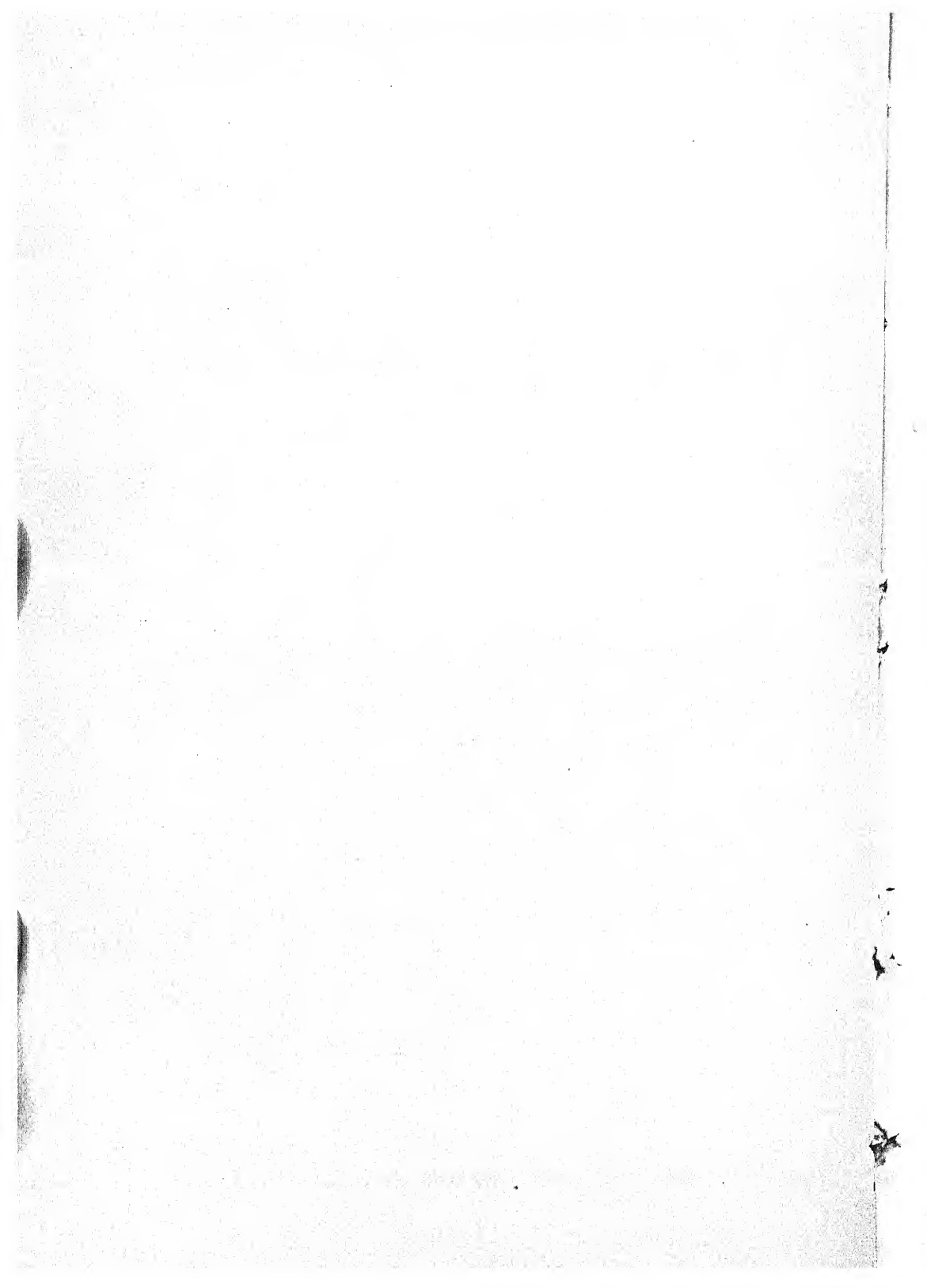
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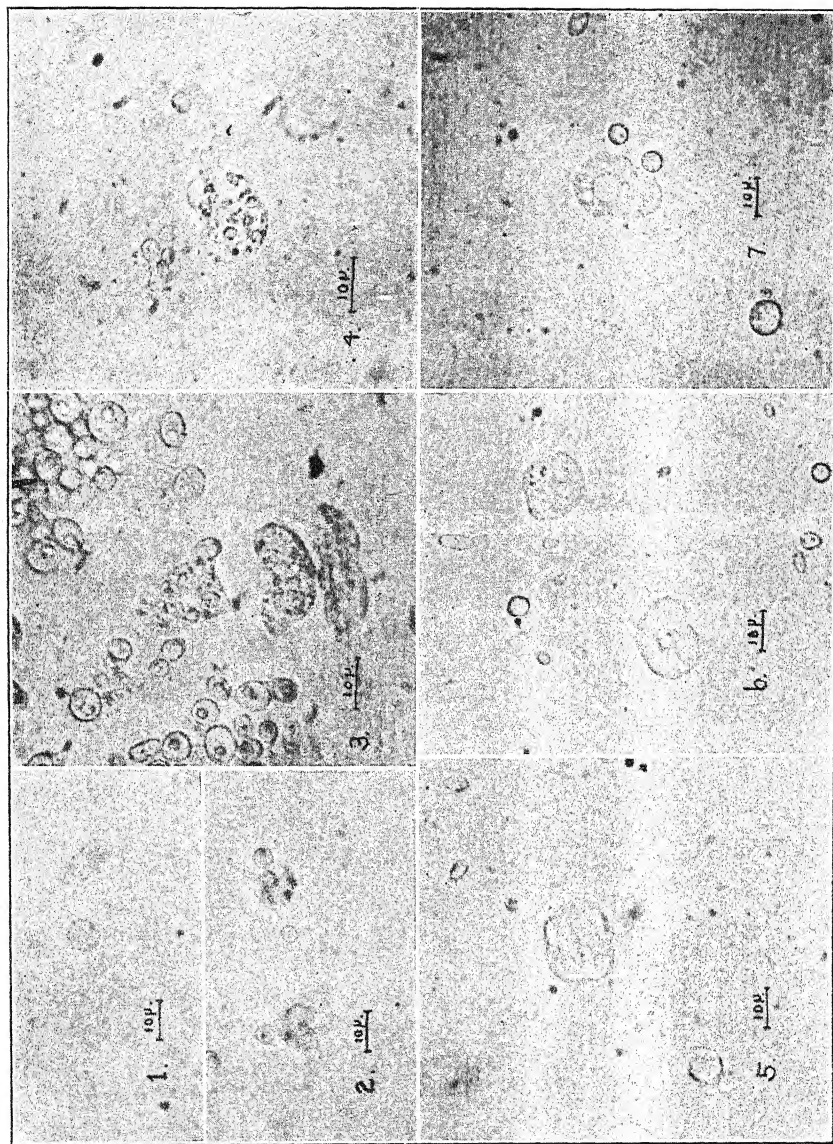
EXPLANATION OF PLATES

PLATE IV

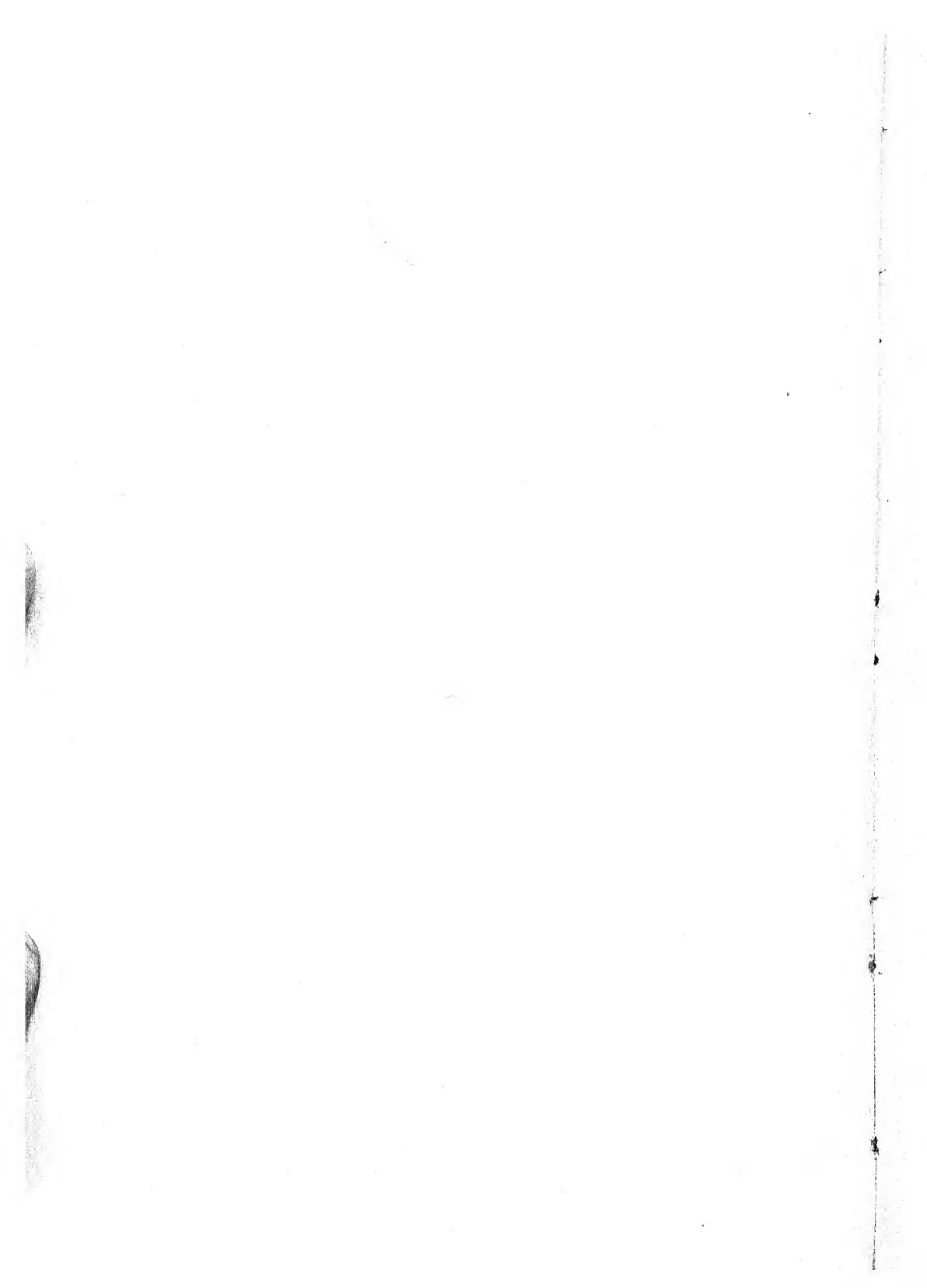
FIGS. 1-6. Stages in the division of a swarm cell containing spores of *Monilia candida* that have been ingested. The spores are not ejected but are retained within the swarmer during the process, as is shown in figures 1-5. Figure 6 shows one of the daughter cells a







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few moments after division, containing two of the four fungous spores originally present in the mother swarm cell.

FIG. 7. Swarm cell with ingested spores of *Trichoderma lignorum*, showing their location in the protoplasm.

FIG. 8. Swarm cell with ingested spores of *Spicaria* sp., one of which was taken in, even though it had put out a germ tube one-and-one-half times its diameter.

FIG. 9. Swarm cell which has taken in a portion of the germ tube of a *Spicaria* spore. In this case, as in others that have been observed, the germ tube was ejected later by the swarm cell.

FIG. 10. Swarm cell with ingested spores of *Monilia candida*.

FIG. 11. A swarm cell which has attached itself to a spore of *Monilia sitophila* too large to be ingested. There was no evidence that the spore was in any way affected by the action of the protoplasm of the swarm cell.

FIG. 12. Swarm cell containing ingested spores of *Candida breve* and showing the form assumed by the swarmer during the creeping movement.

FIG. 13. Swarm cell showing the characteristic form it assumes during the rotating movement.

PLATE V

FIG. 1. Swarm cells shortly after germination. The one at the left has just encysted under the intense heat and light of the lamp while taking the picture; the other has assumed the creeping movement. $\times 420$.

FIG. 2. Swarm cells some time after emergence showing ingested spores of *Monilia candida*. The outline of these spores themselves is not so clear as that of the oil globules which they contain. $\times 420$.

FIG. 3. Three large swarm cells crowded with ingested spores of *Monilia candida* and bacteria. $\times 580$.

FIG. 4. A very large swarm cell filled with ingested spores of *Monilia candida* and bacteria. $\times 580$.

FIGS. 5, 6, 7. Swarm cells with vacuoles containing spores of *Monilia candida* and bacteria in the process of digestion. At this stage these swarm cells still retain their flagella and hence may still be called swarm cells even though their movement is chiefly amoeboid. They will soon pass into the typical myxamoeboid condition. $\times 420$.

CONCERNING THE MORPHOLOGY OF *MICROSTROMA* AND THE TAXONOMIC POSITION OF THE GENUS

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INTRODUCTION

The genus *Microstroma* has been placed at various times among the Basidiomycetes, as an Exobasidiomycete, and among the Fungi Imperfecti, without, as yet, entire agreement as to its correct taxonomic position. The present work on the genus was undertaken with a view toward studying the general morphology, and in favorable material the cytology, to secure if possible further information which might be of assistance in classification.

The genus was first described by Niessl (25) in 1861, who placed it in the Fungi Imperfecti. In 1888, Schröter (31), having carefully studied the structure of *Microstroma*, was struck by the constancy of the number of spores, and by the similarity between its conidiophores and the basidia of the Exobasidii. He consequently placed *Microstroma* among the Exobasidiaceae, in the Basidiomycetes. This position was adopted by P. Hennings (7), Magnus (18), and Patouillard (26), who made *Microstroma* a sub-genus in the genus *Exobasidium*. Recent authors such as Herter (11) and Migula (24) place *Microstroma* in the Exobasidiomycetes.

Maire has published the most detailed recent work on this genus. In 1906 (19), basing his opinion on a histological and cytological study of *M. juglandis* and *M. album*, he states that these fungi should be excluded from the Basidiomycetes. He says in this connection that "the absence of karyogamy from the formation of the pseudobasidia shows that they are in reality only simple conidiophores." Again in 1913 (20) in a paper on the structure and systematic position of *Microstroma* and *Helostroma*, he concludes that *Microstroma* cannot be placed in the Basidiomycetes and that its correct place in the Fungi Imperfecti is in the Melanconiales near *Colletotrichum* and *Cylindrosporium*, rather than in the Mucedineae or Moniliales.

Doctor E. A. Burt in a letter to Professor Stevens in 1922 says: "In 'La Structure et la position systematique des *Microstroma* et *Helostroma*,' R. Maire showed conclusively that these fungi are Hyphomycetes;" and again in 1923, "I regard *Microstroma juglandis* as an Hyphomycete."

MORPHOLOGY OF *MICROSTROMA*

This study has been limited to the species *Microstroma juglandis*, the type species for which the genus was created; *M. album*; *M. juglandis*

var. *robustum*; and *M. pithecolobii*. The first two named are more or less common throughout the United States and many parts of Europe on leaves of *Juglans*, *Quercus*, *Carya*, etc. *M. juglandis* var. *robustum* was first reported by Higgins from Georgia on pecan catkins, and *M. pithecolobii* was collected by F. L. Stevens in Porto Rico and in British Guiana on *Pithecolobium saman*.

The material for the study of *M. album* was taken from Ellis-Everhart's Fungi Columbiana, no. 1270; Sydow's Exotici Exsiccati, no. 153; and Rabenhorst's Fungi Europaei, no. 3387. Material for the study of *M. juglandis* and *M. pithecolobii* was supplied from the herbarium of the University of Illinois. The author is also indebted to Doctor B. B. Higgins who kindly furnished material of both *M. juglandis* and *M. juglandis* var. *robustum* which he had collected in fresh condition, killed, and imbedded in paraffin.

In the case of dried material the usual treatment with lacto-phenol, followed by washing, was used. The material was then run up through the alcohols and through xylol of varying strengths and was finally imbedded in paraffin. Sections were cut 10 μ , 5 μ , and (in the case of those used for cytological study) 2-3 μ thick. Leitz Wetzlar oil-immersion lenses 1/12 and 1/16 were used.

Various combinations of stains were tried. Magdala red and light green and Delafield's haematoxylin and eosin were good for differentiating the mycelial threads from the host tissue. Flemming's triple stain was most satisfactory in cytological work. A combination of fuchsin and Flemming's triple stain was also found to be valuable.

The fungus is found on the under surface of the leaves of the infected tree, forming a white powdery growth. Sections of the diseased leaf show the fungus to have a very definite manner of growth. The mycelium ramifies between the cells of the host tissue, becoming massed together beneath the stomata into relatively large stromata. These hyphae give rise to the basidia which push up through the stomata. The basidia are clavate, as shown in the figures, and have a definite number of sterigmata on each of which is borne a spore.

The species *M. pithecolobii* Lamkey (32) is found on *Pithecolobium saman*. It produces white hypophyllous spots on the leaf, and has the general appearance of a *Ramularia*. The basidia are clavate (Pl. VI, fig. 1), 17-40 μ long, emerging through the stomata in a crowded head; the sterigmata are minute, spores rod-shaped, uninucleate, and hyaline, 2 x 8-10 μ . The mycelium is composed of very fine threads, and is septate and branched. The sub-stomatal stroma is shown in the figure. It measures 29-35 μ deep and 51-68 μ broad.

Microstroma album (Desm.) Sacc. was first described by Desmazières (6) in 1838 as *Fusisporium album*. It was later put in the genus *Microstroma* by Saccardo. In 1902 Patouillard created a new genus, *Helostroma*, for this species.

The material of *M. album* studied showed it to have the structure indicated by Patouillard (27) as that of *Helostroma*. In this case also the mycelium is intercellular and forms stromatic masses under the stomata. The stomata are about $25\ \mu$ thick and give rise to a group of conidiophores which are cylindrical, not clavate as in *M. pithecolobii*, and $25\text{--}35\ \mu$ long. The conidiophores bear at their tips lateral enlargements on which are produced the spores. These enlargements are also found at various distances along the conidiophores. Each bears a packet of 6 or 7 spores. These conidia are hyaline and ellipsoidal and measure $5\text{--}6 \times 2\text{--}3\ \mu$. The fact which Patouillard points out, that the spores are borne on lateral tubercles at various levels on the conidiophores, seems to justify his placing this species in the genus *Helostroma*. Whether or not the *M. album* that was originally described possessed these characters or was truly of the *Microstroma* type could be determined only by examination of original specimens.

M. juglandis (Bérang.) Sacc. (fig. 2) was described as *Fusidium juglandis* in 1847 (2). It was placed in *Microstroma* as *M. leucosporium* by Niessl in 1861 (25) and by Saccardo as *M. juglandis* (30).

In the material studied the intercellular mycelium was not so abundantly scattered among the host cells as in the other species, being rarely found among the palisade cells. The stroma was found to be a very definite structure, however (fig. 7). The individual threads of the mycelium were very fine, averaging about $1\text{--}1.5\ \mu$ in thickness. The stomata were about $45\ \mu$ deep and $35\ \mu$ broad. The basidia pushing up through the stomata were club-shaped or clavate, with usually six sterigmata on which were borne six spores. The basidia were $17\text{--}20\ \mu$ long. The spores, as shown in figure 7, were rod-shaped with rounded ends, $2\text{--}2.19\ \mu$ broad and $5.8\text{--}7.3\ \mu$ long. The single nucleus was very distinct in the center of the spore.

A study of the cytology of this species was also made. Measurements of the cells of the mycelium showed them to average $3.6\ \mu$ long and $1.4\ \mu$ broad, very little larger than many ordinary bacteria (fig. 8). Maire (20) states that the cells of the mycelium "are almost always uninucleate." On the contrary, I have found the nuclei to be associated in pairs. Considering the minute size of the cells the nuclei are necessarily rather small, but with proper staining and high magnification they show as very definite structures (fig. 4). To get an accurate picture of the position of the nuclei it is better to study threads of the mycelium, which are isolated among the host cells, as the threads making up the stroma become so intermingled that even in the thinnest sections the structure cannot be clearly determined.

No mention has been made by previous writers as to the presence or absence of clamp connections between the cells of the mycelium. I have found that such structures, very similar to those described and pictured by Harper in *Hypochnus*, do occur (fig. 6). Other structures, somewhat resembling clamp connections but very much elongated, also occur. They are not ordinary branches as is shown (fig. 8) by the fact that they are

cut off from two cells. This suggests the clamps of Hoffman as he originally applied the term, except for the great elongation of the protuberance.

Microstroma juglandis var. *robustum* was first described by Higgins (12). The appearance and structure of this variety (fig. 5) are very much like that of *M. juglandis* except for the fact that it forms a larger stroma and that the basidia are more numerous and larger, and the spores larger. The original author states that the differences are not great enough to warrant the naming of a new species, the robust habit perhaps being due to the more abundant food supply furnished by the pecan catkins. This form is well described by its author as follows:

Host tissue pale, often slightly distorted; mycelium intercellular, forming more or less dense mats between the host cells; fruiting stromata oval to short conical, 60 to 100 by 55 to 150 μ , composed of very slender interwoven threads; basidia club-shaped, 13 to 30 by 5 μ , bearing apically 6 to 8 spores on short sterigmata; spores hyaline, one-celled, cylindrical, rod-shaped, 9 to 14 by 3 to 5 μ .

DISCUSSION

The Basidiomycetes have long been known as a group of fungi characterized by the fact that they possess typical structures known as basidia, upon which are borne basidiospores. They are further characterized by branched, septate mycelium which is always well developed. De Bary (5) describes the basidia as "generally club-shaped terminal cells of hyphal branches, with spores abjointed at their broad apices, usually at the extremities of long sterigmata." While the number of spores borne by a basidium may vary in the group, there is a marked constancy in the number of spores characteristic of a species or genus. Structures somewhat resembling basidia are found among the Hyphomycetes, as in the Aspergillaceae. In this family the conidiophores are often inflated at the apex and the spores are borne on stalks or sterigmata, but the conidia are catenulate and are not constant in number.

Until the latter part of the nineteenth century, the cytology of fungi had hardly been studied. In 1866, De Bary described nuclei in the reproductive cells of several Ascomycetes and Basidiomycetes. In 1884 he added to his former observations, and in the same year Strasburger described the nuclei in the Agaricineae, including several Psalliotae, Amanitae, and Russulae. In *Russula* he observed a single nucleus in the basidium, and its divisions to form the eight nuclei of the spores. He describes two nuclei as going to each spore.

Rosenvinge (29) worked under Strasburger and found that in the mature or almost mature cells of the Basidiomycetes studied the number of nuclei was generally more than one, but that cells were present which contained only one nucleus. He concluded that in the young cells there was probably only one nucleus, at least in several species, and that in the young basidium there was always a single nucleus. In only one case, *Tricholoma*

virgatum, did he see any indication of indirect division in the basidium. The fungi studied included species of *Corticium*, *Boletus*, *Clavaria*, *Hygrophorus*, *Amanita*, and others.

Maire (22) presented data for Hymenomycetes derived from a study of nine genera of Polyporeae and Agaricineae. He found in every case that the cells of the young carpophore were normally binucleate, and that the pairs of nuclei divided simultaneously as conjugate nuclei. Two nuclei only fused to form the primary nucleus of the basidium, which by two divisions gave rise to the four nuclei of the basidiospores. In a second paper, Maire (21) reported the results of a study of five genera of the Gasteromycetes. He found that in every case the cells of the hymenium contained two nuclei associated in conjugating mitosis, and that only two nuclei fused to form the primary nucleus of the basidium.

Harper (10) in his study of *Hypochnus subtilis* found that the cells of the old hymenium and the cells of the mycelium contained two nuclei each, placed rather closely together. He was unable to observe conjugate division in the formation of new cells in the mycelium. He also studied the nuclear phenomena in *Coprinus ephemerus*, and found the cells of the pileus and stipe to be multinucleate. The cells forming the gills of a carpophore, however, were all binucleate.

In 1913 Levine (16) gave in tabulated form all available data as to the number of nuclei in the cells of the mycelia, rhizomorphs, carpophores, etc., in the Basidiomycetes investigated up to that time. The evidence at present seems to indicate that a binucleate condition is typical of the mycelium and sub-hymenium of the Basidiomycetes, but that there are probably many variations from this condition.

Clamp connections were first described by Hoffman (13). They are formed by outgrowths from the lateral wall of the cell, near the transverse septum. This protuberance elongates and finally reaches the lateral wall of the cell on the other side of the septum. The walls become dissolved so that there is open communication through the tube. A wall is then laid down separating the tube and the cell from which it arose. Sometimes a second wall closes the opening between the tube and the second cell. It was to this exceptional case that Hoffman first applied the term "clamp cells."

Clamp connections have been observed in many Basidiomycetes, and are reported by Harper (10) in *Hypochnus subtilis* and *Coprinus ephemerus*; by Buller in *Coprinus*; and by Levine (16) in various species of *Coprinus* and many other cases. Some forms have been reported which apparently lack clamp connections. Lyman in studying species of *Corticium* found that in *C. subgiganteum* no clamps were produced at all. Fitzpatrick (8) failed to find clamp connections in *Eocronartium musicola*, either on the endophytic hyphae or in the sporophore. He said it was possible, though not probable, that they might be present for only a short time in the young mycelium.

In a report in 1913 Kniep (14) mentioned small bodies in the clamp connections resembling disintegrating nuclei. In his later work (14) he used these as the basis of a new theory as to how binucleate cells arise in the Basidiomycetes. The fungi studied were *Corticium varians* and *C. serum* Pers. The spores of these species are uninucleate as is the mycelium until the formation of clamps, when the nucleus divides. According to the review by Levine (15) the clamp connections are formed in the regular manner. During the process one nucleus, following conjugate division, passes into the clamp, two more to the apical region of the cell, and one to the basal part of the cell. The transverse septum is formed across the cell immediately below where the clamp originated. The clamp is cut off from the first cell, fuses with the second, and the nucleus which is in it passes into the second cell, joining the one there and so completing the pair.

Kniep found this peculiar type of conjugate division also in *Panus stipticus*, *Clitocybe flaccida*, and *Polyporus destructor*. In spite of the fact that these forms have been studied by other cytologists, who failed to find such divisions, this author unfortunately does not give pictures or figures.

The function of clamp connections has been variously interpreted. Brefeld, and later Harper, believed that they had something to do with the circulation of food in the mycelium. R. Hartig and others believed that such cell unions resemble a sexual act.

Miss Bensaude (1) found the same nuclear behavior in connection with the clamps that is given by Kniep, but she went further and called one nucleus + and one -, introducing the idea that there were plus and minus strains in Basidiomycetes similar to those found by Blakeslee (3) in the Mucorales. She accepted Falck's classification of the mycelium into primary, secondary, and tertiary forms. Cross walls with clamp connections never appeared in the hyphae of the primary mycelium. In single-spore cultures of *Coprinus*, Miss Bensaude "found abundant production of mycelium which remained primary, and did not produce carpophores. When parts of each mycelium were mixed in a culture, a secondary mycelium appeared and fruit bodies were produced." According to this author secondary mycelium may revert to primary mycelium, in which case a uninucleated cell appears among binucleated cells. No clamps are found on cross walls of this cell.

Miss Hanna (9) states that the presence of clamp connections associated with conjugate division of the nuclei furnishes a reliable criterion for determining whether a given species of the Hymenomycetes is homothallic or heterothallic. If clamp connections are found on mycelia of single-spore cultures the species must be homothallic; if they are found only when mycelia from two different spores are grown in one culture, the species must be heterothallic.

The Uredinales were placed in the Basidiomycetes as a family by Brefeld (4) in 1888. Not much work of importance was done on the cytology of

the Uredinales until 1895, when Poirault and Raciborski (28) published their results. They described a binucleated condition in many stages and also conjugate division of the pairs. Further study of this group has shown that a binucleated condition exists in the cells of the mycelium and sub-hymenium, and in the young teliospore, fusion of the two nuclei occurring at this point so that the mature teliospore has but one nucleus.

Similar to this analogy between the rusts and the Basidiomycetes is the relationship which has been shown to exist between the smuts and the Basidiomycetes. Dangeard, Tisch, and Schmitz, did some work on the cytology of smuts, and stated that the mycelium was composed of multi-nucleated cells. Lutman (17) found that while this statement might be true of the genus *Ustilago*, it was not true of the Tilletiaceae. He found binucleated cells in *Entyloma Nymphaeae* and reported that in *Urocystis Anemones*, *Doassansia Alismatis*, and *Doassansia deformans* many if not all the cells are binucleated. The fact that two nuclei fuse in the teliospore of the rust and the young basidium was cited. Lutman's results showed that a similar fusion occurs regularly in the chlamydospores of the smuts, and in this he confirmed previous results obtained by Dangeard, Maire, and Raciborski.

CONCLUSIONS

In the discussion of the Basidiomycetes given above, this class is shown to possess as its distinguishing characteristics more or less clavate basidia bearing sterigmata, constant in number, on which are produced single, non-catenulate spores. The occurrence of clamp connections and of binucleated mycelial cells is also found to be commonly associated with the class. The existence of one or more of these three characters would certainly strongly incline one to consider a fungus as a basidiomycete. The occurrence of all three of these characters in the same fungus leaves little room for doubt as to the proper classification.

In *Microstroma juglandis*, as has been shown above, both binucleated mycelium and clamp connections are found. This statement is limited to this one species because of the fact that for it alone was suitable material available for cytological study. In the case of *M. pithecolobii* the cells of the stalks of the basidia contained two structures which stained darkly and gave every indication of being nuclei (fig. 3); however, this material was not in good condition for cytological study, so that the structures are indicated as present but no statement can be made in this case that they really are nuclei. However, in the case of *M. juglandis* there seems to be no question as to the nature of the structures indicated. Since this is the case, little doubt remains as to the proper taxonomic position of this apparently typical species of the genus *Microstroma*; it must be placed with the Basidiomycetes.

Although these results have, at present, been obtained in only one species, there is no reason to question the fact that a study of properly

killed, sectioned, and stained material of the other species of the genus will reveal the existence of similar conditions in each case.

The author is greatly indebted to Professor F. L. Stevens for the suggestion of this problem and for the assistance he has given throughout the investigation.

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EXPLANATION OF PLATE VI

FIG. 1. Cross section of leaf of *Pithecolobium saman* showing stroma of *Microstroma pithecolobii*. $\times 740$.

FIG. 2. Cross section of hickory leaf showing structure of the stroma of *M. juglandis*. $\times 740$.

FIG. 3. Basidia of *M. pithecolobii*. $\times 4000$.

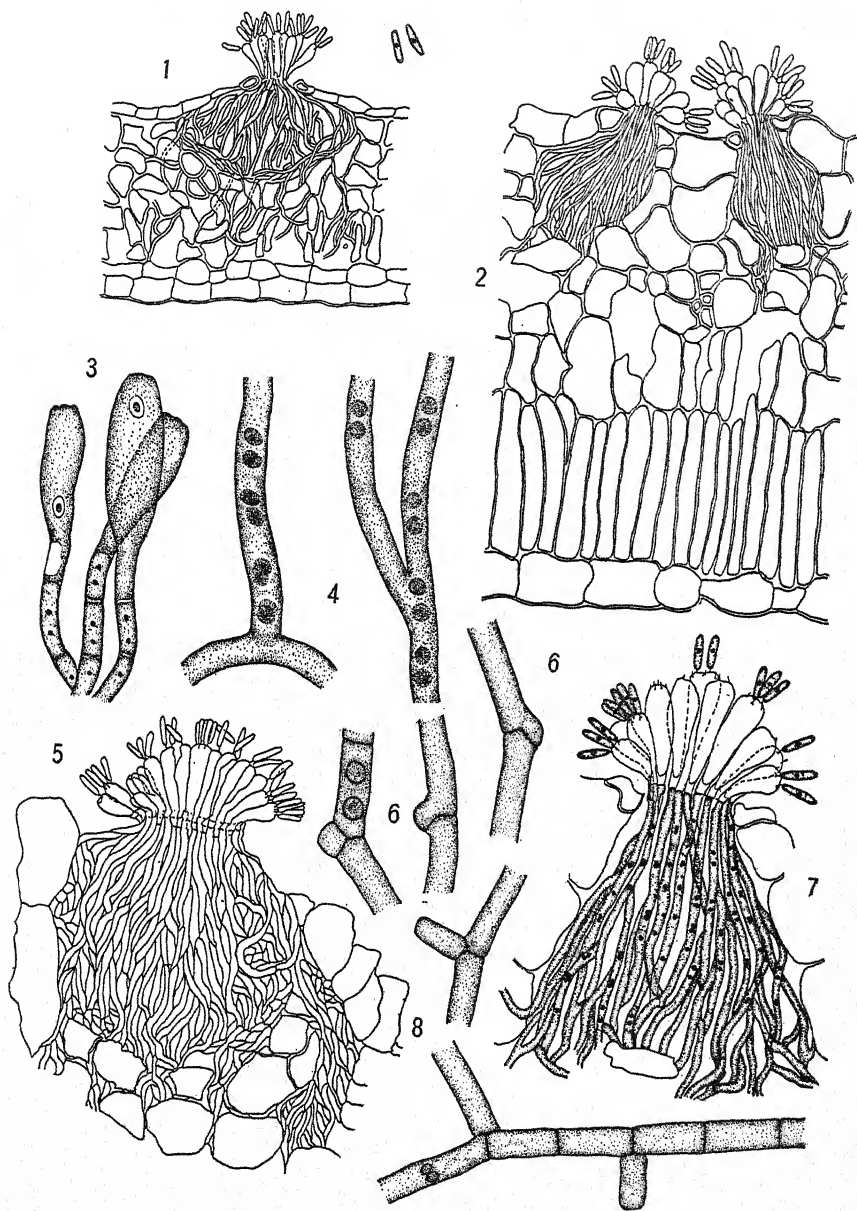
FIG. 4. Binucleated mycelium of *M. juglandis*. $\times 5000$.

FIG. 5. Section of diseased pecan catkin showing the structure of the stroma of *M. juglandis* var. *robustum*. $\times 740$.

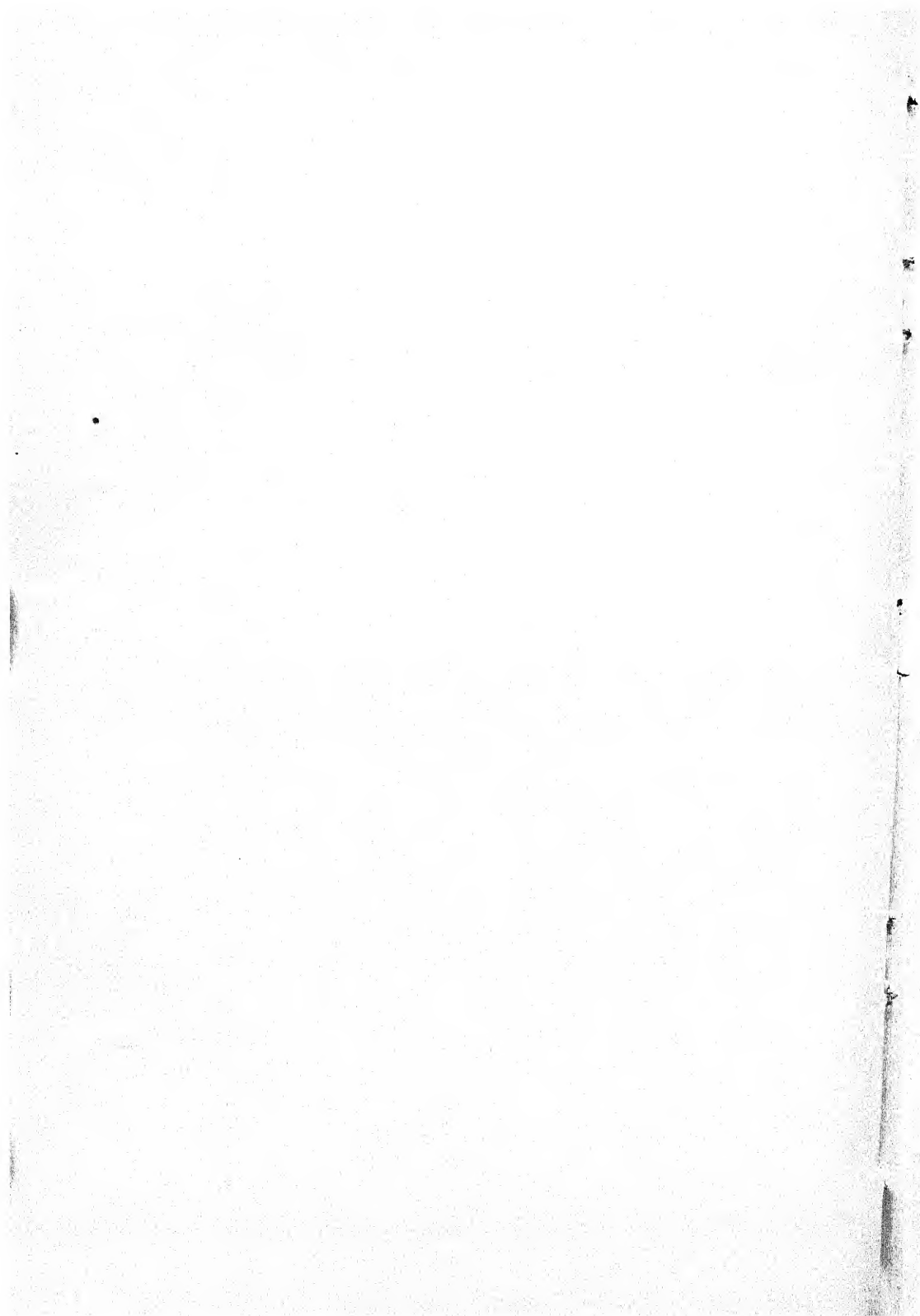
FIG. 6. Typical clamp connections and a binucleated cell of *M. juglandis*. $\times 5000$.

FIG. 7. Section through a stroma of *M. juglandis* on hickory, showing mycelium, basidia, sterigmata, and spores in detail. $\times 1500$.

FIG. 8. Cells of the mycelium of *M. juglandis*; also structures resembling clamp connections but very much elongated. $\times 5000$.



PIRES' MICROSTROMA



MYCORHIZAS FROM NORTH CAROLINA AND EASTERN TENNESSEE

W. B. McDougall

(Received for publication June 21, 1927)

During August, 1926, the writer spent some time studying the mycorrhizal relations of the woody plants in the Great Smoky Mountains of North Carolina and Tennessee, and to a very limited extent also in the vicinity of Wilmington, North Carolina. In addition to observations made in the field, roots were collected from 16 different species, collections being made from several individuals of each species. These roots were later imbedded, sectioned, and studied microscopically.

OBSERVATIONS

No mycorrhizas were found on *Leiophyllum buxifolium*, *Rhododendron maximum*, or *Sassafras variifolium*. *Liriodendron tulipifera* and *Liquidamber styraciflua* were found to have endotrophic mycorrhizas and no detailed studies of these were made. Ectotrophic mycorrhizas were found on *Castanea dentata*, *Betula lutea*, *Magnolia acuminata*, *M. Fraseri*, *Quercus velutina*, *Carya glabra*, *Abies balsamea*, *Picea rubra*, *Tsuga canadensis*, *Pinus virginiana*, and *P. palustris*.

Nine collections from *Castanea dentata*, one from Tennessee and eight from North Carolina, yielded three slightly different forms. All three have filamentous fungus mantles but one of them is slightly rough and fleecy on the outside (text fig. 1), one is smooth (text fig. 2) and the third has hyphae projecting out from the surface like root hairs (text fig. 3). Most of the sections of *Castanea* mycorrhizas contain a greater or less number of cells filled with a substance that is green after the sections have been stained with magdala red and counterstained with light green in clove oil. Sometimes this substance is only in the cells of the endodermis and sometimes farther out in the cortex (text figs. 1, 2, 3). Wherever this deposited substance occurs the fungus seems unable to penetrate beyond the cells containing it.

The one collection from *Betula lutea* was taken on Mt. Le Conte, Tennessee, at an altitude of about 6,000 feet and was apparently caused by a red *Russula*. The sections show a rather thin filamentous fungus mantle which is smooth on the outside. Penetration is between the cells of the outermost row only. These cells are only slightly elongated radially and most of them contain some of the green-staining deposit.

One collection was taken from *Magnolia acuminata* on Mt. Le Conte, Tennessee, at an altitude of about 2,000 feet. The mycorrhizas were white

when fresh and microscopic sections show a fungus mantle similar to that described by McDougall and Jacobs (6) from *Pinus Murrayana* in which the inner part is pseudoparenchymatous and the other part filamentous. In this case a somewhat greater portion of the mantle is filamentous than is true of the *Pinus Murrayana* mycorrhiza. The interior of the *Magnolia* mycorrhiza is of the typical deciduous tree type (McDougall and Jacobs, 6) with radially elongated cells on one side of the root only and penetration of the fungus filaments about half way through the cortex. The green-staining deposit is not much in evidence except in a few cells near the root tips.

One collection from *Magnolia Fraseri* was taken near Yellow Gap in the Pisgah National Forest, North Carolina. The mycorrhizas of this species are similar in general appearance to those of *Magnolia acuminata* but there are two forms. One of these is like that of *M. acuminata* except that it is not so well developed especially as to the penetrating filaments, while the other differs in having all of the fungus tissue filamentous.

Three collections taken from *Quercus velutina* in different parts of the Pisgah National Forest, North Carolina, showed some interesting variations. In one form the fungus tissue is strictly parenchymatous throughout but the mantle, which is relatively thick and smooth on the outside, consists of two parts, the outer of which is composed of rather large cells and the inner of much smaller cells. In this form the outermost cortical cells are radially elongated on all sides of the root and they constitute the greater part of the cortex, the remainder consisting of only two layers of rather small cells. The fungus penetrates between the radially elongated cells and sometimes a little farther but never clear to the endodermis, which is filled with the green-staining deposit. Other specimens from the same collection are similar to the one described except that they have the more usual occurrence of the radially elongated cells on one side only, and still others show no radially elongated cells at all. In these latter specimens there is a considerable amount of the green-staining deposit in the outer cortical cells and the fungus has scarcely penetrated the cortex at all. One of the other collections showed the usual deciduous tree form with the fungus mantle filamentous and very loose and fleecy on the outside.

Two collections from *Carya glabra* in the Pisgah National Forest yielded three forms of ectotrophic mycorrhizas. One of these shows the typical deciduous-tree type with rather thin filamentous mantle, only slightly roughened on the outside, and good penetration into the cortex. The green-staining deposit is absent. The second form, from the same collection, is similar except that the mantle is distinctly parenchymatous and the endodermis is filled with the green-staining deposit. The third form, from the second collection, has a filamentous mantle that consists of two parts, the inner part being very compact and the outer rather loose and with many hyphae projecting from the surface. The inner compact part stained

well with Magdala red while the outer looser portion did not take the stain well, so that the two portions differ considerably in color on the finished slides. None of the cortical cells in this form are elongated to any great extent and the fungus does not penetrate very far. The endodermis is completely filled with the deposit.

Six collections were made from *Abies balsamea* on Mt. Le Conte, Tennessee. The first, third, and sixth collections appear by both macroscopic and microscopic examinations to be the same although each was taken under a different kind of mushroom. The first was taken under a pink *Russula*, the third under a *Spathularia*, and the sixth under a diseased *Boletus*. Since *Russula* was rather common under the balsam trees it seems likely that all of these mycorrhizas were caused by the same species of *Russula*. The mantle on these mycorrhizas is pseudoparenchymatous and smooth on the outside. Internally penetration is nearly to the central cylinder, though usually not quite, and some specimens show the peculiar condition of deep penetration on one side of the root and penetration to only a short distance on the other side. A deposit appearing to be the same as that noted in the mycorrhizas of deciduous trees is present in some of the cells of these balsam roots. The second collection from balsam was taken under a fruit body of *Amanitopsis vaginata* and the specimens appeared greenish when fresh. The slides made from them were not well finished but they seemed to be morphologically similar to those described above. The specimens of the fourth collection were light bluish when fresh while those of the fifth collection were white. Morphologically the two are alike but quite different from those of the other four collections. The mantle is distinctly filamentous, not very thick, and rather loose and fleecy on the outside. Penetration in most cases extends to the central cylinder.

One collection from *Picea rubra* on Mt. Le Conte yielded specimens with a thin and loose filamentous mantle. There is a considerable amount of deposit in the cortical cells and the fungus seems to have had considerable difficulty in penetrating the cortex.

One small collection supposedly from *Tsuga canadensis* in the Pisgah National Forest shows mycorrhizas of the deciduous tree form. Since this apparent exception to the usual condition in the Coniferae suggests a possible error in making the collection, it would not be safe to base any conclusions on this one collection and any further remarks concerning the mycorrhizas of the hemlock will therefore be reserved until there has been an opportunity for further study of this species.

One collection from *Pinus virginiana* in the Pisgah National Forest yielded mycorrhizas of a common coniferous tree form with pseudoparenchymatous mantle which is smooth on the outside and shows penetration of the fungus to the central cylinder. The endodermis is filled with the green-staining deposit.

Two collections were made from *Pinus palustris* in the vicinity of

Wilmington, North Carolina. The mycorrhizas of this species are similar to those of *Pinus virginiana* in general morphological form but the mantle is often very thin and penetration into the cortex often slight. No case was found in which the fungus had penetrated clear through the cortex. The cells of the cortex as well as those of the endodermis are largely filled with the green-staining deposit.

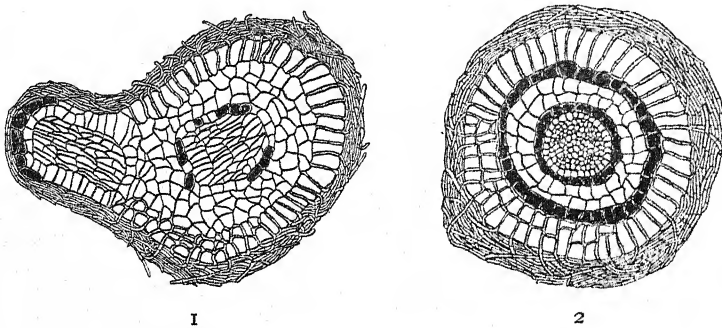
DISCUSSION

The month of August, 1926, during which the mycorrhizas described in this paper were collected, marked the end of a dry period of about two years duration in the Pisgah National Forest, North Carolina. The rains started in the latter part of July and continued, with showers nearly every day, for a period of several weeks. Thus at the time the collecting was done the weather was ideal for the growth of mushrooms and it was very evident that an abundant crop was being produced. Previous to this time, however, the conditions for mushroom growth had been unfavorable for about two years and the forest rangers and other natives stated that mushrooms had not been in evidence during that time. When mycorrhizal fungi are not actively growing no mycorrhizas are produced and it might be expected, therefore, that very few if any mycorrhizas were produced in the Pisgah National Forest during this prolonged dry period. This is believed to have been the case since there were very few of the old dead mycorrhizas which are usually abundant in deciduous forests. It was quite evident, too, that the new crop of mycorrhizas was just being produced while the collecting was going on. They were not well developed on most trees when collecting was started early in August but became increasingly more abundant later. On the other hand, there was no evidence that any of the trees had suffered any ill effects from the dry weather or the absence of mycorrhizas during the two preceding years. Forest trees are relatively immune from the effects of minor fluctuations in soil moisture conditions because of their extensive and deeply penetrating root systems so that, although this dry period had been so prolonged that there had been no water in any of the mountain streams for months, the deeper roots of the trees had undoubtedly been in constant communication with adequate supplies of water. Much more important for our present argument, however, is the fact that the trees did not suffer from the absence of mycorrhizas during this long period, since it is contended by Melin (3) and others that the mycorrhizal fungi benefit the trees by supplying them with necessary nitrogen compounds. Undoubtedly if the drought were sufficiently prolonged the trees would suffer eventually; but they would suffer from lack of water and not from lack of mycorrhizas. Thus, the evidence here is in favor of the contention of Masui (1), McDougall (4, 5), and McDougall and Jacobs (6) that the ectotrophic mycorrhizas of forest trees are beneficial to the fungi but not to the trees.

McDougall and Jacobs (6) have described a form of mycorrhiza from *Pinus Murrayana* in which the fungus mantle is composed of two layers, the inner being pseudoparenchymatous and the outer filamentous. Masui (2) has described a somewhat similar condition in a mycorrhiza of *Alnus firma*, and on *Alnus japonica* he found one in which the mantle was entirely filamentous but consisted of three layers, the outer layer being composed of very small filaments, the middle layer of large filaments, and the inner layer of filaments with dense cell contents. He also found that in very young portions of these mycorrhizas the mantle is very thin and the layers are not differentiated but that secondary thickening of the middle and inner layers takes place as the mantle matures. The present paper describes a form from *Magnolia acuminata* similar to that described by McDougall and Jacobs; one from *Quercus velutina* that differs from any previously described in that the mantle is entirely parenchymatous but the outer portion is composed of large cells and the inner of small ones, and one from *Carya glabra* in which the filamentous mantle has an inner compact part that stains dark red and an outer loose part that stains pale red with magdala red. These findings bring up the question as to whether these dimorphic and trimorphic mantles are really due to polymorphic variations of the same fungus or whether more than one fungus is concerned in the formation of such a mantle. Masui found three species of fungi concerned in the production of mycorrhizas on *Alnus firma* var. *Sieboldiana*, one with a black mycelium, one with a white, and one with a yellow. In several cases he found the black mycelium overlapping the white or the yellow and thus forming an outer secondary mantle. In this case the distinction between the kinds of fungi was easy because of the differences in color, but in case there were no differences in color the distinction would be very difficult. It seems not impossible, therefore, that most if not all cases of polymorphic mantles are due to the presence of more than one mycorrhizal fungus. The question is an important one because it has been usual to consider each of several morphological forms of mycorrhizas to be caused by a specifically distinct fungus.

It is rather common to find some of the root cells of ectotrophic mycorrhizas, especially the cells of the endodermis, filled with a solid substance. This deposited substance is unusually abundant in some of the North Carolina material and certain observations on it have caused it to assume a position of considerable importance in the consideration of the symbiotic relation of the mycorrhizal fungus to the tree. Practically every section of *Castanea* mycorrhizas examined showed some cells containing this deposit. Usually it is found in some of the cells of the endodermis and at the extreme tip of the rootlet, as shown in text figure 1. If this were the only condition represented it would not call for much comment. Text figure 2, however, shows a more significant arrangement. Not only is every cell of the endodermis filled but there is a layer of cells in the cortex,

midway between the central cylinder and the outside of the root, every cell of which is filled with this deposit, and it will be noted that the fungus has penetrated as far as this layer of cells and no farther. Again, text



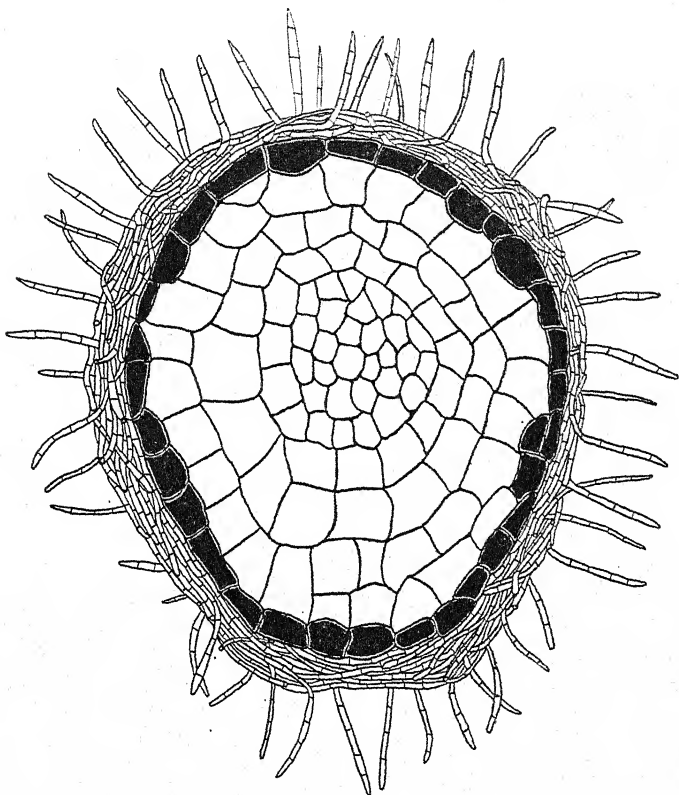
TEXT FIG. 1. Section of mycorrhiza of *Castanea dentata* showing a moderate amount of deposit in the endodermis and in cells at the tip of a lateral branch.

TEXT FIG. 2. Section of mycorrhiza of *Castanea dentata* showing the endodermis and one row of cells in the cortex filled with a deposit. The fungus has not been able to penetrate the tissue containing the deposit.

figure 3 shows an even more significant condition in that the epidermal or outermost cortical layer of cells contains the deposit and nowhere has the fungus penetrated the root. It appears that the fungus is unable to pass beyond cells containing this deposit and it seems likely that the substance has been secreted by the root as a protection against the invading parasite. Various other modifications in the position and abundance of the deposit were observed but in no case in a *Castanea* root was the fungus found extending beyond cells containing the deposit. In other kinds of mycorrhizas the deposit is frequently present in greater or less amounts but often does not appear to be quite such an effective bar against the penetration of the fungus. In *Quercus velutina* and *Carya glabra* the deposit is usually only in the endodermis and the fungus does not get that far. In the pines, the balsam fir, and the birch the deposit is more in evidence, and while the fungus in many cases pushes in between cells containing the deposit it usually appears to be checked by the presence of this substance. This is especially noticeable in one specimen of *Abies balsamea* in which the deposit is much more abundant toward one side of the root than the other with a corresponding deep penetration on one side and penetration to a short distance on the other side.

Attempts to identify this deposit have so far not met with success. It was at first thought that it might be a tannic substance but it has not been possible to obtain a blue color with either ferric chlorid or ferric acetate. It has been suggested that it may be a glucoside but its failure to dissolve in hydrochloric acid casts doubt upon this hypothesis. Of course it is

quite possible that the substance has been changed chemically or physically, or in both ways, during the processes of fixing and dehydrating. It is



TEXT FIG. 3. Section of mycorrhiza of *Castanea dentata* showing the outermost layer of root cells filled with the deposit. The fungus has not been able to penetrate the root at all.

hoped that an opportunity may be had soon to investigate this material further in fresh *Castanea* roots.

SUMMARY

Studies were made during August, 1926, of the mycorrhizal relations of 16 species of woody plants of North Carolina and Tennessee. No mycorrhizas were found on *Leiophyllum buxifolium*, *Rhododendron maximum*, or *Sassafras variifolium*. *Liriodendron tulipifera* and *Liquidamber Styraciflua* were found to have endotrophic mycorrhizas, while *Castanea dentata*, *Betula lutea*, *Magnolia acuminata*, *M. Fraseri*, *Quercus velutina*, *Carya glabra*, *Abies balsamea*, *Picea rubra*, *Tsuga canadensis*, *Pinus virginiana*, and *P. palustris* possessed ectotrophic mycorrhizas.

In the Pisgah National Forest, where most of the collecting was done,

the two preceding years had been very dry. The dry period ended in the latter part of July and was followed by a period of several weeks during which there were rains nearly every day. Because of these conditions dead mycorrhizas of the previous year were almost entirely lacking and new mycorrhizas were just developing. No evidence was found, however, that any of the woody plants had suffered any ill effects either from lack of water or from lack of mycorrhizas.

The fact that some forms of mycorrhizas possess fungus mantles that consist of two or more layers differing morphologically from one another suggests that in some cases more than one species of fungus may be concerned in the production of a mycorrhiza.

An unidentified deposit, frequently present in the root cells of ectotrophic mycorrhizas, was found to be unusually abundant in the mycorrhizas of *Castanea dentata*. In no case was the mycorrhizal fungus observed to penetrate into the root past cells containing this deposit, and it is believed that the deposit is produced by the root as a protective reaction against the invading mycorrhizal fungus.

The data presented in this paper support the hypothesis that in the ectotrophic mycorrhizas of forest trees the symbiotic relationship between the fungus and the higher plant is antagonistic and not reciprocal; that is, that the fungus is a parasite on the root and derives food from it but that the root receives no benefit and may be injured by the relationship.

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HIGH PRESSURE AND SEED GERMINATION¹

P. A. DAVIES

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INTRODUCTION

This paper deals primarily with the direct application of high pressure to seeds. Certain physico-chemical effects of high pressure have been discussed in an earlier paper (Davies, 4).

De Vries (3), using rather low pressures (6 to 8 atmospheres) for a period of 2 to 3 days, found in one species, *Oenothera Cockerelli*, that only a 2 percent germination was obtained in 5 days before applying the pressure, while after applying the pressure a 72-percent germination was obtained in 3 days. Bulgia (1), working with caryopses of *Hordeum vulgare*, found that reduced oxygen pressure retarded the activity of the caryopses; while Morinaga (7, 8), with seeds of *Trifolium repens* and *Typha latifolia*, found an increased germination under reduced oxygen pressure.

The present study deals with the application of high pressure to seeds of *Medicago sativa* (alfalfa) and *Melilotus alba* (sweet clover). The delay or failure to germinate in the two species studied is due entirely to the seed-coat, for when the impermeable nature of the seed-coat is destroyed either by the application of hydraulic pressure or by other mechanical means, the seeds germinate readily. There are two mechanical methods already in use by which the percentage germination of hard seeds may be increased: first, by scarification, through the blowing of the seeds against coarse sand-paper or some other abrasive; and secondly, by the use of a strong corrosive such as concentrated sulfuric acid.

Harrington (5) reports very favorably on the practice of scarification, but Nelson (9) says that with the larger types of seeds (red clover), although the percentage of "hard" seeds is reduced, the increased number of broken seedlings results in a total germination as low as the untreated seeds or lower. Concentrated sulfuric acid has been used by many workers to increase the percentage germination of hard-coated seeds. Love and Leighty (6) found with the seeds of *Melilotus alba* that treatments from 25 minutes to an hour resulted in good germination tests, but that a treatment for 2 hours resulted in injury to the seeds. Coe and Martin (2) obtained a good germination test from the seeds of *Melilotus alba* after 15 minutes treatment with the acid.

For the application of high pressure to be advantageous, the seeds must retain the possibility of a high percentage of germination after being dried

¹ From the Laboratory of General Physiology, Harvard University.

for a definite period. This is just what has been found to happen: the seeds used not only retain the possibility of a high percentage of germination, but the percentage germination increases and remains high for a definite period after the seeds have been dried.

METHODS

The chamber used for the pressure experiments was a large alloy-steel cylindrical block one foot in diameter with a three-quarter-inch hole through the center. The walls of the block about the openings were tapped so that a plug could be screwed in each opening, closing the chamber. Through one of the plugs ran an inlet from an ordinary high-pressure hand pump. The two pressures applied were 500 and 2000 atmospheres. The cups for holding the seeds were made of pyrex glass, and were of such a diameter that they fitted loosely in the pressure chamber. After the lower plug had been screwed into the alloy-steel block, the cups containing the seeds (approximately 3000 seeds were placed in each cup) were lowered in place. Four cups could be placed in the chamber at one time. The chamber was filled with water and the other plug screwed into place. The pressure was then applied.

All seeds were germinated on moist filter paper in sterile petri-dishes in a germinator at 20° C. After 3 days in the germinator the initial count was made and the germinating seeds discarded. The final count was made the fifth day. The total germination comprises the sum of the 3-day and the 5-day counts. Seeds that were saved for future tests were dried on glass plates in the sunlight. After the seeds appeared thoroughly dry, they were stored in sealed envelopes until needed for testing. Germination tests were made immediately after the pressure was applied, after 30 days, after 6 months, and finally after 10 months.

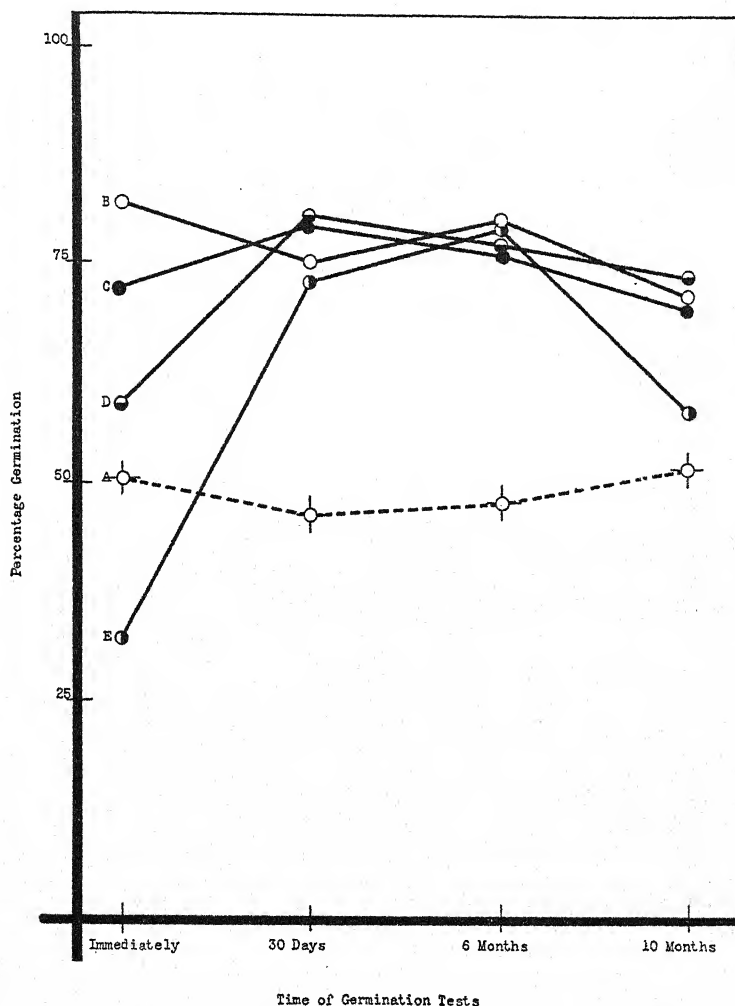
DISCUSSION OF RESULTS

Text figure 1 shows the effect of a pressure of 2000 atmospheres at $18^{\circ} \pm 2^{\circ}$ C. on germination of seeds of *Medicago sativa*. Tested immediately after the pressures were applied, seeds exposed for 1, 2, and 5 minutes show total germinations² above the total germination of the control.³ The exposure for 1 minute was the best, followed by the 2- and the 5-minute exposures. The total germination after 10 minutes exposure fell considerably below the total germination of the control. After 30 days, increases in total germination are observed in seeds exposed for 2, 5, and 10 minutes, with 10-minute exposure the best. Comparing the results after 30 days with those obtained immediately after the pressures were applied, a greater increase in total germination is observed in seeds exposed

² Each test represents the average of two germination tests from the same treated bulk.

³ The controls were untreated seeds from the same bulks as the treated seeds, saved and germinated at the same time as the treated seeds.

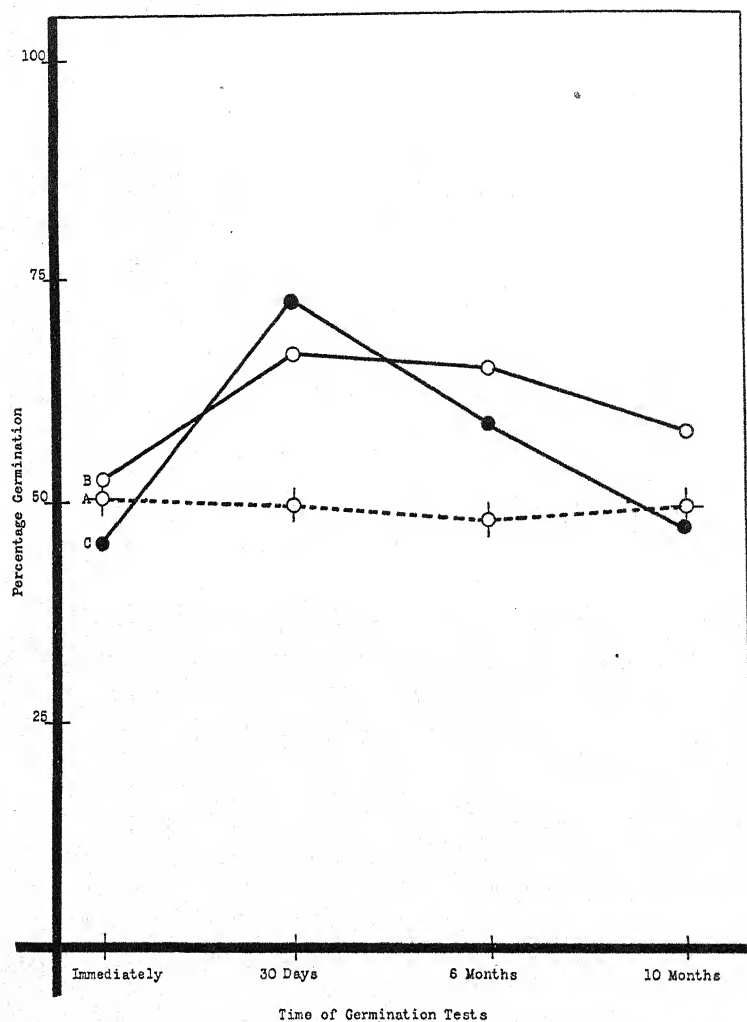
for 5 and 10 minutes. This seems to indicate that injury is suffered by the embryos in the long exposures to 2000 atmospheres, and that there is a partial recovery when the seeds are dried and germinated after 30 days.



TEXT FIG. 1. The effect of 2000 atmospheres pressure at $18^{\circ} \pm 2^{\circ}$ C. on germination of seeds of *Medicago sativa*. Curve A (broken line) represents the total germination of the control; B, C, D, and E, exposures for 1, 2, 5, and 10 minutes, respectively.

The 6-months tests gave practically the same results as were obtained in the 30-days tests. The results obtained in the 10-months tests for exposures of 1, 2, and 5 minutes were nearly the same as those obtained in tests after 30 days and after 6 months, but the exposure for 10 minutes shows a sudden drop.

Text figure 2 shows the effect of a pressure of 500 atmospheres at $18^{\circ} \pm 2^{\circ}$ C. on germination of seeds of *Medicago sativa*. In tests made

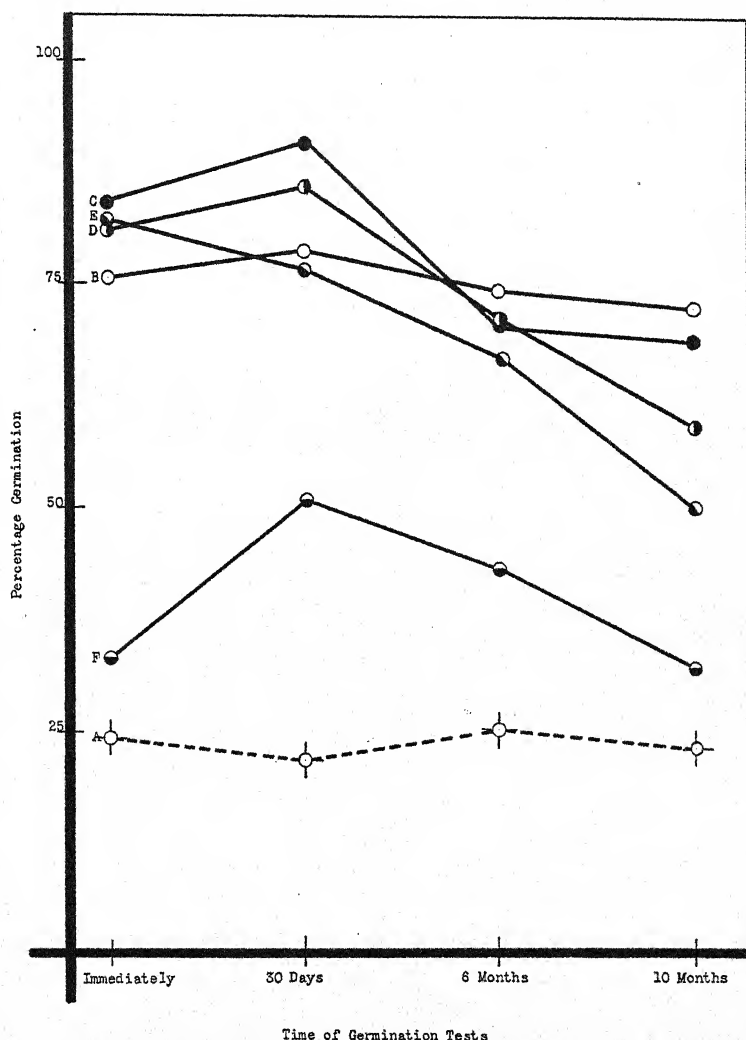


TEXT FIG. 2. The effect of 500 atmospheres pressure at $18^{\circ} \pm 2^{\circ}$ C. on germination of seeds of *Medicago sativa*. Curve A (broken line) represents the total germination of the control; B, exposure for 2 hours; C, exposure for 8 hours.

immediately after the pressures were applied, the total germinations were practically the same as the total germination of the control. Increases in total germination are noticeable in the 30-day tests, and particularly in the 8-hour exposure. In the 6-months and the 10-months tests the 8-hour exposure failed to give as high total germination as the 2-hour exposure. Comparing text figures 1 and 2, the data show that shorter exposures (1 to

5 minutes) at 2000 atmospheres gave higher total germinations than longer exposures (2 to 8 hours) at 500 atmospheres.

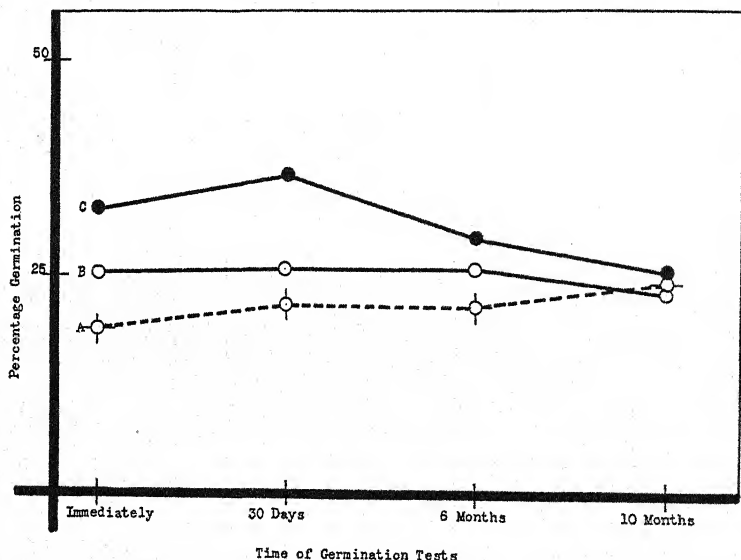
Exposing seeds of *Medicago sativa* to 2000 atmospheres at 0° C. for 5 minutes gave a total germination, when germinated immediately after the pressure was applied, of 71.25 percent. This is nearly identical with the 2-minutes exposure as shown in text figure 1. There appears to be no striking beneficial effect derived from exposing seeds to pressure at low temperatures, for shorter exposures at ordinary room temperature will give just as good results.



TEXT FIG. 3. The effect of 2000 atmospheres pressure at $18^{\circ} \pm 2^{\circ}$ C. on germination of seeds of *Melilotus alba*. Curve A (broken line) represents the total germination of the control; B, C, D, E, and F, exposures for 5, 10, 15, 20, and 30 minutes, respectively.

Text figure 3 shows the effect of a pressure of 2000 atmospheres at $18^{\circ} \pm 2^{\circ}$ C. on germination of seeds of *Melilotus alba*. In the tests made immediately after the pressures were applied, the results were nearly constant in the exposures for 10, 15, and 20 minutes. The 5-minute exposure fell slightly below, while the 30-minute exposure fell far below, the 10-, 15-, and 20-minute exposures. The 30-day tests show slight increases in germination in seeds exposed for 5, 10, and 15 minutes. The total germination of seeds exposed from 5 to 20 minutes was lower in the 6-months and the 10-months tests than in the tests made immediately after the pressures were applied and after 30 days. The low total germination in the seeds exposed for 30 minutes, although greater than the total germination of the controls, is due to the injurious effect of the pressure.

Text figure 4 shows the effect of a pressure of 500 atmospheres at



TEXT FIG. 4. The effect of 500 atmospheres pressure at $18^{\circ} \pm 2^{\circ}$ C. on germination of seeds of *Melilotus alba*. Curve A (broken line) represents the total germination of the control; B, exposure for 2 hours; C, exposure for 8 hours.

$18^{\circ} \pm 2^{\circ}$ C. on germination of seeds of *Melilotus alba*. No striking increases in total germination are shown in the seeds exposed to pressure. Comparing the results shown in these tests with those shown in text figure 3, it is obvious that shorter exposures (5 to 15 minutes) to 2000 atmospheres gave greater total germination than longer exposures (2 to 8 hours) to 500 atmospheres.

Seeds of *Melilotus alba* exposed to a pressure of 2000 atmospheres at 0° C. for 30 minutes and then tested immediately gave a total germination of 62.29 percent. This, although above the total germination of the control,

is below the 5-minute exposure as shown in text figure 3. These results confirm the results shown for *Medicago sativa*, that no beneficial results are obtained in exposing seeds to pressure at 0° C.

SUMMARY

1. The application of a pressure of 2000 atmospheres at $18^{\circ} \pm 2^{\circ}$ C. to seeds of *Medicago sativa* increased the total germination over 50 percent when the seeds were allowed to dry and were germinated after 30 days and after 6 months.

2. The application of a pressure of 2000 atmospheres at $18^{\circ} \pm 2^{\circ}$ C. to seeds of *Melilotus alba* for from 5 to 20 minutes increased the total germination over 200 percent when the seeds were dried and stored for 30 days before the germination tests were made. An increase of over 150 percent in germination was obtained between 5 and 20 minutes exposures with samples from the same bulks exposed to the same pressure for the same periods but in which the seeds were dried for 6 months and for 10 months before the germination tests were made.

3. The 2-hour and 8-hour exposures to 500 atmospheres at $18^{\circ} \pm 2^{\circ}$ C. failed to produce the increase in germination that resulted from 1-minute to 10-minute exposures with *Medicago sativa* and 5-minute to 20-minute exposures with *Melilotus alba* when the seeds were exposed to 2000 atmospheres pressure. Short exposures at high pressures are more advantageous than long exposures at low pressures.

4. Seeds of *Medicago sativa* exposed to 2000 atmospheres pressure at 0° C. for 5 minutes and germinated immediately after the pressure was applied gave a lower total germination than seeds exposed for 1 minute at $18^{\circ} \pm 2^{\circ}$ C.; and seeds of *Melilotus alba* exposed for 30 minutes under the same conditions approximate the 5-minute exposure at $18^{\circ} \pm 2^{\circ}$ C. Short exposures at room temperature are just as advantageous for germination as long exposures at 0° C.

The writer is greatly indebted to Professor P. W. Bridgman of the Jefferson Physical Laboratory of Harvard University for suggesting this problem, and for the application of the pressures to the seeds.

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PHYSIOLOGIC RACES OF BUNT OF WHEAT¹

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The discovery of physiologic races in covered smut of barley (2, 4) and in the loose and covered smuts of oats (6, 8) has greatly stimulated the study of host specialization in the other cereal smuts. From this standpoint, these parasites have previously been a neglected group, although physiologic specialization has long been known to be of wide occurrence among the parasitic fungi (5). This neglect is due, in part at least, to the fact that most collections of smut spores are capable of infecting a wide range of hosts, and thus relatively few varieties are of value in differentiating specialized races. Another factor in the situation is due to the peculiar mode of infection of the smuts. In most of them entrance of the fungus occurs through the young seedling by means of germinating smut spores present on the seed or in the adjacent soil. Various environal conditions such as soil temperature, soil moisture, etc., play a very large part in determining whether infection will occur and, unless the proper combination of conditions is present, very low percentages of infection take place and quite variable results are obtained with equally susceptible varieties.

Faris (3), in his studies on the influence of various factors on the infection of wheat by *Tilletia laevis* Kühn and *T. tritici* (Bjerk.) Winter, obtained some evidence of a difference in the infection capacity of collections of bunt from different localities. In his experiments he used six collections of each smut, testing them on ten different varieties of winter wheat. The most definite evidence of specialization was observed in the reaction of the variety Kanred to certain collections of *T. tritici*.

The writer has for several years studied the behavior of wheat varieties to both species of bunt (*Tilletia laevis* and *T. tritici*) although very little of the accumulated data has been published (7). However, collections of both species have been obtained from widely separated localities and tried out on many varieties of wheat. For the most part, winter varieties have been grown, and by far the larger number of those tested have been more or less severely infected by practically all the collections of bunt. A few varieties have, however, shown marked differences in their behavior to some of the bunt collections, and thus furnish striking evidence of the existence of physiologic races in both the species of *Tilletia*. A brief

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statement concerning these findings has previously been published (9). The evidence obtained showed clearly that both species of bunt contain highly specialized races which are distinguished by their capacity for infecting certain varieties. Rodenhiser and Stakman (10) have also recently presented some evidence of physiologic specialization.

In my experiments the most useful varieties for differentiating these races are Martin, Hussar, Odessa, Kanred, and Turkey. Some of these have been found to be remarkably resistant to *Tilletia tritici* by Stephens and Woolman (12) whose results have been confirmed by more extensive tests in Washington, Oregon, and California (13). Briggs (1) has studied the reaction of hybrids involving Martin or Hussar as parents resistant to his collection of *T. tritici*, and has obtained interesting results on the inheritance of the resistant quality.

INFLUENCE OF ENVIRONMENTAL CONDITIONS ON BUNT INFECTION

Mention has already been made of the profound influence of environmental conditions on infection. A large number of investigators have published data on the influence of temperature, soil moisture, and the nature of the substratum on the infection of wheat by *Tilletia tritici* and *T. laevis*, these results having been reviewed in my previous paper (7). Faris (3), in his experiments with these two smuts, has added new data on several of the problems.

In general, it has been found that low temperatures are most favorable for the infection of susceptible wheats by both species of bunt. At temperatures much above 10° C. the percentages of infection fall off very rapidly and very few seedlings grown at temperatures higher than 20° C. are infected. Moisture is also recognized as playing an important rôle. Some data have also been obtained on the influence of the substratum, Faris usually securing higher infections in potting soil than in sand.

A large amount of data on the influence of the date of planting has been obtained, although the results have been somewhat conflicting. In general, however, late-sown fall or winter wheat is more severely infected than early-sown. On the other hand, early-sown spring wheat gives a higher percentage of infection, as compared with late-sown. However, the soil temperature, soil moisture, etc., at the time of sowing are the determining factors, and it may very well happen that these conditions are more favorable at the time of early seeding of fall wheat than at the later period.

Securing favorable conditions for infection is a primary necessity in the study of varietal resistance and the possible occurrence of physiologic races and, on a large scale, is not a problem that is easy of solution. In general, we may rely, in the case of winter wheat, on late fall plantings, and in my own experiments the seeding of the varieties has been postponed until late in October. However, we have to meet with the danger of severe winter-killing on account of the plants not having become sufficiently established,

a result which actually occurred in my experiments in 1925-1926. A more reliable method is to make duplicate plantings on two or three dates in the fall. This involves, of course, considerable extra labor, but it gives a better chance of obtaining the most favorable conditions for good infection.

The best method of solving the difficulty, however, is to germinate the inoculated seed under carefully controlled conditions. In this way the most favorable combination of temperature, moisture, substratum, etc., may be secured. This method involves the necessity of transplanting the seedlings, and, if large numbers of plants are involved, the task becomes a very serious one.

RESULTS FOR 1924-1925 AND 1925-1926

In the fall of 1924, eight collections each of *Tilletia laevis* and *T. tritici* were sown on seventy varieties of winter wheat. Three additional collections of *T. laevis* and one of *T. tritici* were sown on thirty varieties and six collections of *T. laevis* and three of *T. tritici* were sown on ten varieties. The varieties used for the most part were the common winter ones which are grown throughout the winter wheat belt of the United States. In general, very high percentages of infection were obtained, varying somewhat among the different varieties.

Conclusive evidence as to the existence of specialized races was secured. One collection of *T. laevis* (no. 1) stood out sharply from the others in its capacity to infect Martin and Odessa. Collection no. 5 seemed to be significant in its marked capacity for infecting Kanred. In a similar manner, two collections of *T. tritici* (nos. 2 and 3) gave high infections with Hussar, Martin, and Odessa. On the other hand, nos. 1, 6, and 8 were negative on Turkey, Hussar, Martin, and Odessa.

In 1925-1926, eight collections of *Tilletia laevis* and ten of *T. tritici* were used on fifteen varieties of winter wheat. These were selected on the basis of the previous year's work, either being very susceptible to all the collections of smut or very resistant to some, while susceptible to others. Unfortunately, the weather after planting was very unfavorable and a poor stand resulted. Consequently the number of plants obtained the following season was unusually small in many cases. However, clear-cut indications of specialization were secured, the results for 1924-1925 in the main being extended and confirmed.

RESULTS WITH *TILLETIA LAEVIS*, 1926-1927

In the fall of 1926, spores of nine collections of *Tilletia laevis* were sown on eighteen varieties of winter wheat and twelve additional collections on ten varieties. The varieties used were some of those that were included in the experiments in the previous years. Several of the varieties—Fulcaster (255), Fultz (256), Harvest Queen (258), Mealy (260), and Mediterranean (261)—were originally obtained from the Farm Crops Department of the

TABLE 1. Summary of Results with *Tilletia laevis* Kühn

	Seed No.	Prague, Czechoslovakia	Vienna, Austria	Asparn, Austria	Barl, Italy	Bologna, Italy	Bologna, Italy	Egypt	Tunis	South Africa	Tarascon, France	Tarascon, France	Alaugh, France	St. Cannot, France	St. Cannot, France	Bouilles, France	Lambesc, France	St. Victoret, France	Piedmont, Missouri	Xenia, Ohio	Albany, New York	Ithaca, New York
Dawson.....	713	4	7	10	3	7	9	5	4	9	3	6	5	6	6	4	9	6	5	8	6	6
Fulcaster.....	255	0	3	3	1	3	1	0	1	4	0	0	4	8	8	5	8	3	2	3	3	0
Fultz.....	256	5	8	9	5	9	7	7	3	8	8	0	4	—	—	6	10	10	5	8	5	7
Harvest Queen.....	258	6	7	—	8	9	9	6	6	7	5	5	7	9	8	4	10	9	8	6	7	9
Leap.....	314	6	—	—	4	—	—	9	1	7	2	—	—	—	—	—	—	—	8	8	4	0
Mealy.....	260	9	—	—	5	—	—	5	4	—	5	—	—	—	—	—	—	—	6	8	6	6
Mediterranean.....	261	7	10	10	9	10	10	6	7	10	7	5	—	—	9	7	9	10	8	8	8	10
Poole.....	319	4	—	—	1	—	—	1	1	—	1	—	—	—	—	—	—	—	2	3	3	3
Red Wave.....	352	0	—	—	0	—	—	0	1	—	0	—	—	—	—	—	—	—	7	10	0	0
Kanred.....	716	0	7	3	0	2	3	0	1	0	0	—	—	—	—	—	—	—	6	8	8	8
".....	717	1	—	—	1	—	—	0	1	—	0	—	—	—	—	—	—	—	9	10	3	10
Turkey.....	729	0	10	4	0	0	0	0	0	0	0	0	0	7	7	8	2	1	7	3	0	0
Hussar.....	527	0	4	0	0	0	0	0	0	0	0	0	0	—	—	0	0	0	1	0	0	0
".....	814	0	—	—	—	—	—	0	0	0	0	—	—	—	—	0	—	—	0	0	0	0
Martin.....	724	3	4	4	0	0	0	0	0	0	0	—	—	—	—	0	0	—	0	0	0	0
Odessa.....	726	7	—	—	0	—	—	0	0	0	0	—	—	—	—	0	0	—	0	0	0	0
".....	727	7	5	2	0	—	—	0	0	0	0	0	0	—	—	0	0	—	0	1	0	0

* The figures at the top of the columns are the numbers of the collections.

— = No trial.

0 = No infection.

1 = 1-10 percent.

2 = 11-20 percent.

3 = 21-30 percent.

4 = 31-40 percent.

5 = 41-50 percent.

6 = 51-60 percent.

7 = 61-70 percent.

8 = 71-80 percent.

9 = 81-90 percent.

10 = 91-100 percent.

TABLE 2. *Results with Tilletia laevis Kühn*

	Seed No.	Year	Laevis No. 1 Czechoslovakia			Laevis No. 10 Vienna, Austria			Laevis No. 5 Piedmont, Mo.			Laevis No. 2 Albany, N. Y.		
			No. Plants	No. Inf.	Percent Inf.	No. Plants	No. Inf.	Percent Inf.	No. Plants	No. Inf.	Percent Inf.	No. Plants	No. Inf.	Percent Inf.
Dawson.....	713	1925	17	16	94.1	—	—	—	21	15	71.4	27	10	37.0
		1926	16	13	81.2	—	—	—	20	16	80.0	12	5	41.6
		1927	25	10	40.0	35	22	62.8	28	14	50.0	26	15	57.6
Fulcaster.....	255	1925	20	12	60.0	—	—	—	19	9	47.3	14	11	78.5
		1926	—	—	—	—	—	—	17	14	82.3	—	—	—
		1927	21	0	0	23	6	26.0	28	3	10.7	24	5	20.8
Fultz.....	256	1925	26	24	92.3	—	—	—	19	14	73.6	26	25	96.1
		1927	34	14	41.1	—	—	—	38	19	50.0	35	28	80.0
Harvest Queen...	258	1925	25	24	96.0	—	—	—	23	21	91.3	35	32	91.4
		1927	19	9	47.3	28	22	78.5	34	26	76.4	17	8	47.0
Leap.....	314	1925	10	9	90.0	—	—	—	—	—	—	15	8	53.3
		1927	30	17	56.6	22	14	63.6	24	21	87.5	28	18	64.2
Mealy.....	260	1925	22	16	72.7	—	—	—	15	11	73.3	12	10	83.3
		1926	—	—	—	—	—	—	10	9	90.0	—	—	—
		1927	10	6	60.0	—	—	—	34	24	70.5	22	15	68.1
Mediterranean...	261	1925	—	—	—	—	—	—	17	11	64.7	28	24	85.7
		1927	36	29	80.5	—	—	—	24	13	54.1	27	22	81.4
Poole.....	319	1925	—	—	—	—	—	—	—	—	—	11	8	72.7
		1927	24	15	62.5	24	23	95.8	26	23	88.4	35	28	80.0
Red Wave.....	352	1925	19	13	68.4	—	—	—	17	6	35.2	31	23	74.1
		1927	22	8	36.3	—	—	—	37	6	16.2	30	9	30.0
Kanred.....	312	1925	—	—	—	—	—	—	6	3	50.0	13	1	7.6
		1926	16	0	0	—	—	—	26	23	88.4	30	4	13.3
		1927	32	0	0	—	—	—	27	17	62.9	36	0	0
".....	716	1925	15	2	13.3	—	—	—	20	16	80.0	27	7	25.9
		1926	10	0	0	—	—	—	15	9	60.0	11	0	0
		1927	22	0	0	24	16	66.6	21	17	80.9	32	0	0
Turkey.....	717	1925	23	15	65.2	—	—	—	38	19	50.0	34	17	50.0
		1926	9	2	22.2	—	—	—	21	10	47.6	14	4	28.5
		1927	23	2	8.6	—	—	—	29	2	6.8	24	0	0
".....	729	1925	5	4	80.0	—	—	—	10	2	20.0	19	0	0
		1926	3	1	33.3	—	—	—	18	0	0	22	0	0
		1927	32	0	0	26	25	96.1	16	0	0	31	0	0
Hussar.....	527	1925	7	1	14.2	—	—	—	17	0	0	18	1	5.5
		1926	6	0	0	—	—	—	13	0	0	7	0	0
		1927	29	0	0	28	11	39.2	35	0	0	18	0	0
".....	814	1925	15	4	26.6	—	—	—	24	9	37.5	27	0	0
		1926	—	—	—	—	—	—	11	0	0	13	0	0
		1927	29	0	0	—	—	—	31	0	0	24	0	0
Martin.....	724	1925	12	5	41.6	—	—	—	13	0	0	12	0	0
		1926	15	8	53.3	—	—	—	18	0	0	22	0	0
		1927	26	6	23.0	23	9	39.1	25	0	0	27	0	0
Odessa.....	726	1927	20	13	65.0	—	—	—	16	0	0	19	0	0
".....	727	1925	—	—	—	—	—	—	7	2	28.5	10	0	0
		1927	22	14	63.6	8	4	50.0	26	0	0	15	0	0

Missouri Agricultural Experiment Station; Dawson (or Honor) (713) was secured from the Cornell Agricultural Experiment Station, Hussar (527) from the Illinois Agricultural Experiment Station, and Kanred (716) and Turkey (717) from the Nebraska Agricultural Experiment Station. The other varieties were secured at various times from the Office of Cereal

Investigations, United States Department of Agriculture. The Cereal Investigations numbers of these are as follows: Hussar (814) C. I. 4843, Kanred (312) C. I. 5146, Leap (314) C. I. 4823, Martin (724) C. I. 4463, Odessa (726) C. I. 4651, Odessa (727) C. I. 4655, Poole (319) C. I. 1979, Turkey (729) C. I. 1571c.

Approximately twenty-five or more plants of each variety were grown to maturity, and the number was frequently in excess of thirty. Sometimes the number fell below twenty due to various causes.

The results with *Tilletia laevis* are given in tables 1 and 2. Table 1 shows, in the form of a summary, the results with all twenty-one collections. Table 2 gives the detailed results for the one or more years of the collections that stand out significantly in their specialization.

Dawson, Harvest Queen, Leap, Poole, and Fulcaster were tested with all twenty-one collections. Poole consistently gave the highest percentages of infection, frequently more than 90 percent being obtained; Harvest Queen and Leap ranked next, followed very closely by Dawson. The Fulcaster variety gave very variable results, usually low percentages of infection resulting. This behavior was quite in contrast with that of the previous years, since Fulcaster usually gave high percentages with all collections of smut used. Fultz, Mealy, Mediterranean, and Red Wave were used with nine collections, and the first three usually gave high percentages of infection. Red Wave, also in contrast to its behavior in previous years, gave rather low results. With the exception of the results obtained with Red Wave and Fulcaster, the data are in harmony with those secured in previous years. All these varieties must rank as very susceptible and, so far as the accumulated data are concerned, very susceptible to all collections of *Tilletia laevis* which have been used.

It is with the varieties Hussar, Kanred, Martin, Odessa, and Turkey that the differences in the behavior of the bunt collections stand out most distinctly. Hussar (527), Martin (724), Odessa (727), Kanred (716), and Turkey (729) were inoculated with the spores of all the different twenty-one collections; while Hussar (814), Odessa (726), Kanred (312), and Turkey (717) were included in the experiments with only nine collections.

Martin (724) was infected with three collections—nos. 1, 10, and 19. With no. 1, 6 out of 26 plants (23 percent) were infected; with no. 10, 9 out of 23 plants (39.1 percent), and with no. 19, 9 out of 28 plants (32.1 percent). In 1925 no. 1 gave 5 infected plants out of 12 (41.6 percent) and, in 1926, 8 infected plants out of 15 (53.3 percent). Only these three collections seemed capable of infecting this variety.

Odessa (726) was severely attacked by *laevis* no. 1, 13 out of 20 plants (65 percent) being infected. It has not been tested with *laevis* nos. 10 and 19. Odessa (727) gave 14 infected plants out of 22 (63.6 percent) with no. 1, 4 out of 8 with no. 10, and 1 out of 26 with no. 3. *Laevis* no. 5 gave positive results in 1925 but negative in 1927.

Hussar (527) was infected by only one collection, namely, no. 10, a total of 28 plants being grown, of which 11 (39.2 percent) were smutted. In all the other series this variety gave negative results. *Laevis* no. 19 in 1925 gave 5 infected plants out of 12 (41.6 percent), but negative results in 1927. Hussar (814), wherever tested, also gave negative results, but, unfortunately, was not included in the series with *laevis* no. 10. Hussar stands out as very resistant to practically all collections, only two, nos. 10 and 19, showing any marked capacity for infecting it, although both strains were slightly infected with no. 1 in 1925.

Kanred (716) was attacked by thirteen of the collections, and very severely by nos. 5, 10, 12, 13, and 14. It proved to be very resistant to no. 1, both in 1927 and 1926, but in 1925, 2 out of 15 plants were smutted. *Laevis* no. 5 has consistently severely infected it all three years. Kanred (312) corresponded very closely, the outstanding features being its resistance to no. 1 and its high susceptibility to no. 5 in all three years.

Turkey (717) was moderately susceptible to nos. 1, 5, and 6. In previous years it gave high percentages with these and also with no. 2. Turkey (729) was severely infected by no. 10, 25 smutted plants out of 26 (96.1 percent) being obtained. No. 19 also gave 9 infected plants out of 26 (34.6 percent). It was negative to no. 1 in 1927 although infected plants in a small total occurred in 1925 and 1926. It was entirely resistant to no. 2 all three years and nearly so to no. 5.

RESULTS WITH *TILLETIA TRITICI*, 1926-1927

Nine collections of *Tilletia tritici* were used on eighteen varieties, and twelve collections on nine varieties (tables 3 and 4). The varieties Dawson, Harvest Queen, Leap, Poole, and Fulcaster were used with all the different collections and the first four named varieties consistently gave very high percentages of infection, it being the exception to find as few as 50 percent smutted plants. The variety Fulcaster gave moderately high infections, but sometimes the percentage was low, and occasionally negative. Fultz, Mealy, Mediterranean, and Red Wave were used with only nine collections, and usually the percentage of infection was high. The variety Red Wave gave somewhat lower percentages than the other three. These nine varieties, with the exception of Fulcaster and Red Wave, gave results corresponding very closely with those obtained in previous years. In 1925 and 1926, wherever the experiments were carried out, much higher percentages were usually secured with both Fulcaster and Red Wave.

Hussar (527) was tested with all twenty-one collections. Severe infections occurred in the experiments with nos. 2, 3, 15, and 16, and occasionally infected plants were secured with nos. 4 and 21. With no. 2, 8 infected plants out of 27 (29.6 percent) resulted; with no. 3, 9 plants out of 29 (31 percent); with no. 15, 20 plants out of 29 (68.9 percent), and with no. 16, 16 plants out of 35 (45.6 percent). *Tritici* no. 21 gave one infected

TABLE 3. Summary of Results with *Tilletia tritici* (Bjerk.) Winter

	Seed No.	Prague, Czechoslovakia	Vienna, Austria	Klagenfurt, Austria	Klagenfurt, Austria	Waltsee, Austria	Halle, Germany	West Seneca, New York	Davis, California	Pullman, Washington	Corvallis, Oregon	Cowra, New South Wales	Seneca, New York	Noisy de Roi, France	Noisy de Roi, France	Bologna, Italy	England	Wye, England	Croston, England	England	Aberystwyth, Wales	Halle, Germany
		2*	3	15	16	17	21	4	1	6	8	7	5	10	11	12	9	14	18	19	20	22
Dawson.....	713	8	10	9	10	10	9	9	9	9	8	6	7	10	10	6	6	9	9	8	7	10
Fulcaster.....	255	8	2	6	6	10	5	4	2	1	1	1	7	7	3	2	1	2	2	2	0	7
Fultz.....	256	8	9	—	—	—	—	9	10	4	6	6	9	7	7	2	5	—	—	—	—	—
Harvest Queen.....	258	9	8	8	10	10	7	10	10	8	6	10	7	10	9	6	8	8	8	10	7	9
Leap.....	314	8	9	9	9	9	—	10	10	7	9	7	8	9	5	—	—	—	—	—	—	—
Mealy.....	260	9	9	—	—	—	—	10	6	9	9	7	9	—	—	—	—	—	—	—	—	—
Mediterranean.....	261	8	10	—	—	—	—	10	8	9	9	9	6	9	—	—	—	—	—	—	—	—
Poole.....	319	10	9	10	10	9	6	10	10	8	10	10	6	9	9	9	8	10	7	9	10	10
Red Wave.....	352	8	10	—	—	—	—	5	8	7	3	4	9	—	—	—	—	—	—	—	—	—
Kaured.....	312	9	7	—	—	—	—	4	2	0	1	3	9	—	—	—	—	—	—	—	—	—
".....	716	10	7	10	10	0	—	7	1	9	3	2	0	8	0	1	1	1	0	0	0	0
Turkey.....	717	0	4	—	—	—	—	3	1	2	0	2	4	—	—	—	—	—	—	—	—	—
".....	729	9	10	—	—	—	—	0	0	0	0	2	0	—	—	—	—	—	—	—	—	—
Hussar.....	527	3	4	7	5	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
".....	814	4	7	—	—	—	—	2	0	0	0	0	0	0	0	—	—	—	—	—	—	—
Martin.....	724	3	6	9	3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Odessa.....	726	9	8	—	—	—	—	9	0	0	0	0	0	0	0	—	—	—	—	—	—	—
".....	727	9	8	—	—	0	0	7	0	0	0	0	2	0	0	—	—	—	—	—	—	—

* The figures at the top of the columns are the numbers of the collections.

— = No trial. 2 = 11-20 percent. 5 = 41-50 percent. 8 = 71-80 percent.
 0 = No infection. 3 = 21-30 percent. 6 = 51-60 percent. 9 = 81-90 percent.
 1 = 1-10 percent. 4 = 31-40 percent. 7 = 61-70 percent. 10 = 91-100 percent.

plant out of 24, and *tritici* no. 4 gave 3 infected plants out of 31 (9.6 percent). This collection also infected the variety in 1925, 2 out of 13 plants (15.3 percent) being smutted, but negative results were secured in 1926. *Tritici* no. 5 gave 8 infected plants out of 13 in 1925 but negative results in 1926 and 1927.

TABLE 4. *Results with Tilletia tritici* (Bjerk.) Winter

	Seed No.	Year	Tritici 2 Prague, Czechoslovakia			Tritici 4, West Seneca, N. Y.			Tritici 1 Davis, Calif.		
			No. Plants	No. Inf.	Percent Inf.	No. Plants	No. Inf.	Percent Inf.	No. Plants	No. Inf.	Percent Inf.
Dawson.....	713	1925	18	14	77.7	24	15	62.5	16	13	81.2
		1926	33	29	87.8	16	10	62.5	18	16	88.8
		1927	33	26	78.7	31	25	80.6	32	26	81.2
Fulcaster.....	255	1925	21	19	90.4	22	19	86.3	19	11	57.8
		1926	14	7	50.0	—	—	—	—	—	—
		1927	22	17	77.2	26	8	30.7	20	3	15.0
Fultz.....	256	1925	27	25	92.5	25	21	84.0	10	7	70.0
		1927	35	25	71.4	36	31	86.1	37	35	94.5
Harvest Queen.....	258	1925	30	30	100.0	—	—	—	17	16	94.1
		1927	33	28	84.8	37	34	91.8	36	33	91.6
Leap.....	314	1925	13	13	100.0	14	7	50.0	—	—	—
		1926	—	—	—	—	—	—	—	—	—
		1927	18	14	77.7	31	29	93.5	29	27	93.1
Mealy.....	260	1925	15	13	86.6	18	18	100.0	12	6	50.0
		1926	20	16	80.0	—	—	—	—	—	—
		1927	32	27	84.3	40	38	95.0	28	25	89.2
Mediterranean.....	261	1925	27	24	88.8	26	22	84.6	13	10	76.9
		1927	28	20	71.4	32	29	90.6	20	16	80.0
Poole.....	319	1925	—	—	—	10	10	100.0	—	—	—
		1927	32	30	93.7	35	34	97.1	29	29	100.0
Red Wave.....	352	1925	14	9	64.2	20	10	50.0	21	10	47.6
		1927	37	28	75.6	28	14	50.0	24	18	75.0
Kanred.....	312	1925	15	11	73.3	13	2	15.3	12	1	8.3
		1926	29	19	65.5	17	2	11.7	7	5	71.4
		1927	25	22	88.0	31	12	38.7	30	4	13.3
".....	716	1925	20	12	60.0	18	8	44.4	24	1	4.1
		1926	20	17	85.0	15	1	6.6	7	5	71.4
		1927	31	29	93.5	28	19	67.8	22	1	4.5
Turkey.....	717	1925	29	17	58.6	21	12	57.1	26	16	61.5
		1926	16	11	68.7	28	9	32.1	29	9	31.0
		1927	26	0	0	26	7	26.9	30	3	10.0
".....	729	1925	9	2	22.2	6	3	50.0	22	0	0
		1926	20	6	30.0	31	1	3.2	26	0	0
		1927	30	25	83.3	35	10	28.5	32	0	0
Hussar.....	527	1925	6	3	50.0	13	2	15.3	17	1	5.8
		1926	14	3	21.4	18	0	0	29	0	0
		1927	27	8	29.6	31	3	9.6	29	0	0
".....	814	1925	21	15	71.4	24	11	45.8	18	0	0
		1926	10	2	20.0	29	0	0	14	0	0
		1927	33	13	39.3	37	5	13.5	29	0	0
Martin.....	724	1925	12	2	16.6	9	2	22.2	10	0	0
		1926	23	5	21.7	24	0	0	20	0	0
		1927	33	10	30.3	26	0	0	35	0	0
Odessa.....	726	1927	20	18	90.0	26	21	80.7	23	0	0
".....	727	1925	13	9	69.2	10	2	20.0	—	—	—
		1927	20	17	85.0	26	16	61.5	16	0	0

TABLE 4 (continued). Results with *Tilletia tritici* (Bjerk.) Winter

	Seed No.	Year	Tritici 5 Seneca, N. Y.			Tritici 10 Noisy de Roi, France			Tritici 9 England		
			No. Plants	No. Inf.	Percent Inf.	No. Plants	No. Inf.	Percent Inf.	No. Plants	No. Inf.	Percent Inf.
Dawson.....	713	1925	—	—	—	26	20	76.9	—	—	—
		1926	22	18	81.8	19	13	68.4	—	—	—
		1927	34	23	67.6	36	35	97.2	32	17	53.1
Fulcaster.....	255	1925	29	20	68.9	16	12	75.0	—	—	—
		1926	8	7	87.5	15	4	26.6	—	—	—
		1927	31	19	61.2	29	19	65.5	22	1	4.5
Fultz.....	256	1925	28	14	50.0	24	17	70.8	—	—	—
		1927	33	28	84.8	—	—	—	23	11	47.8
Harvest Queen.....	258	1925	32	25	78.1	24	4	16.6	—	—	—
		1927	25	16	64.0	28	26	92.8	30	23	76.6
Leap.....	314	1925	16	14	87.5	18	13	72.2	—	—	—
		1926	—	—	—	11	11	100.0	—	—	—
		1927	28	22	78.5	17	14	82.3	36	26	72.2
Mealy.....	260	1925	30	22	73.3	20	15	75.0	—	—	—
		1926	—	—	—	11	10	90.9	—	—	—
		1927	28	24	85.7	—	—	—	23	22	95.6
Mediterranean.....	261	1925	33	22	66.6	28	16	57.1	—	—	—
		1927	30	27	90.0	—	—	—	35	28	80.0
Poole.....	319	1925	9	5	55.5	13	8	61.5	—	—	—
		1927	36	32	88.8	19	17	89.4	25	20	80.0
Red Wave.....	352	1925	31	23	74.1	34	16	47.0	—	—	—
		1927	36	32	88.8	—	—	—	37	22	59.4
Kanred.....	312	1925	15	5	33.3	26	10	38.4	—	—	—
		1926	21	12	57.1	12	0	0	11	0	0
		1927	29	25	86.2	—	—	—	29	0	0
".....	716	1925	—	—	—	26	1	4.1	—	—	—
		1926	20	4	20.0	15	0	0	11	0	0
		1927	32	0	0	31	22	70.9	24	1	4.1
Turkey.....	717	1925	—	—	—	30	6	20.0	—	—	—
		1926	30	10	33.3	23	5	21.7	12	4	33.3
		1927	36	12	33.3	—	—	—	21	2	9.5
".....	729	1926	26	0	0	16	0	0	29	1	3.4
		1927	27	1	3.7	32	0	0	35	0	0
Hussar.....	527	1925	13	8	61.5	16	1	6.2	—	—	—
		1926	15	0	0	12	0	0	12	0	0
		1927	30	0	0	37	0	0	33	0	0
".....	814	1926	20	0	0	15	0	0	19	0	0
		1927	27	0	0	—	—	—	33	0	0
Martin.....	724	1926	28	0	0	18	0	0	22	0	0
		1927	29	0	0	31	0	0	20	0	0
Odessa.....	726	1927	10	0	0	—	—	—	17	0	0
".....	727	1927	25	3	12.0	—	—	—	6	0	0

Hussar (814) was infected only with collections nos. 2, 3, and 4. No. 2 gave 13 infected plants out of 33 (39.3 percent); no. 3, 18 out of 27 (66.6 percent) and no. 4, 5 out of 37 (13.5 percent). These results correspond very closely with those obtained in 1925 and 1926. In 1925 *tritici* no. 2 gave 15 out of 21 plants (71.4 percent) and no. 3, 6 out of 18 plants (33.3 percent). In 1926, no. 2 gave 2 infected plants out of 10 (20 percent) and no. 3, 4 out of 16 (25 percent).

Martin (724) was used in the experiments with all the collections. High infections were obtained with nos. 2, 3, 15, and 16, results being as follows: no. 2, 10 infected plants out of 33 (30.3 percent); no. 3, 21 out of 38 (55.2 percent); no. 15, 25 out of 29 (86.2 percent); no. 16, 9 out of 35 (25.7 percent). No. 2 gave positive results in 1925 and 1926 and no. 3 gave positive results in 1925 but negative in 1926. With *tritici* no. 17, 1 plant out of 35 and with no. 21, 1 plant out of 28 were also found infected. *Tritici* no. 4 gave infections in 1925 on Martin, but the results in 1926 and 1927 were negative.

Odessa (727) was tested with all twenty-one collections and gave positive results with nos. 2, 3, 4, and 5. With no. 2, 17 infected plants out of 20 (85 percent); no. 3, 12 out of 17 (70.5 percent); no. 4, 16 out of 26 (61.5 percent), and no. 5, 3 out of 25 (12 percent) were secured. The results with *tritici* nos. 2, 3, and 4 confirmed those obtained in 1925, *tritici* no. 5 not being tested previously.

Odessa (726) was infected with the same collections as Odessa (727). No. 2 gave 18 out of 20 plants (90 percent); no. 3, 20 out of 25 plants (80 percent); no. 4, 21 out of 26 plants (80.7 percent).

Kanred (716) was very severely infected by a number of collections—nos. 2, 3, 4, 6, 10, 15, and 16. It proved negative with nos. 5, 11, 17, 18, 19, 20, 21, and 22, although it gave positive results with no. 5 in 1926. The remaining collections gave very low percentages of infection.

Kanred (312) was somewhat similar in its behavior to Kanred (716). It was very strongly negative to collection no. 6 obtained from Washington. This was also true in 1926, although in 1925 one infected plant out of 10 was secured. This variety was also negative to no. 10, but gave positive results in 1925.

Turkey (729) was severely infected with collections nos. 2, 3, 15, and 16. With no. 2, 25 plants out of 30 (83.3 percent); no. 3, 31 out of 32 (96.8 percent); no. 15, all 24 (100 percent); no. 16, 22 out of 31 (70.9 percent) were infected. The results with nos. 2 and 3 confirmed those secured in 1925 and 1926.

Turkey (717) was tested with only nine collections, but was severely infected by nos. 3, 4, 5, and 6 and slightly by no. 1. While the results with no. 2 were negative in 1927, evidences of high susceptibility were obtained in 1925 and 1926.

DISCUSSION AND CONCLUSIONS

The behavior of all the collections of both *Tilletia laevis* and *T. tritici* on the varieties Dawson, Fultz, Harvest Queen, Leap, Mealy, Mediterranean, and Poole was essentially the same, no obvious differences in the resistance of these varieties to any collection being observable. Fulcaster and Red Wave gave variable and somewhat low results in the experiments of 1927. In previous years, however, high percentages of infection were obtained with practically all of the collections of the smuts which were used.

It is with the varieties Kanred, Turkey, Hussar, Martin, and Odessa that the differences in behavior of the smut collections are brought out. It is evident from the capacity of the collections to infect these different varieties that physiologic specialization occurs among them.

Tilletia laevis. So far as this species is concerned, at least four specialized races appear to be distinctly differentiated.

Race I is based on the reaction of collection no. 1. It stands out clearly by virtue of its capacity for infecting Martin and Odessa. In 1925 and 1926 Turkey was also severely infected. On the other hand, Kanred and Hussar gave negative results in 1926 and 1927, although a few plants of each of these varieties were smutted in 1925. The material was originally secured from Dr. F. Bubák, Prague, Czechoslovakia.

Race II is based on collection no. 10, and is characterized by its vigorous infection of Kanred, Turkey, Hussar, Martin, and Odessa. Collection no. 19 seems to be identical with it. Both of these were secured from Dr. L. Hecke, Vienna, Austria.

Race III is based on collection no. 5. It is characterized by producing high infections of Kanred and Turkey (717). Turkey (729), Hussar, and Odessa gave a few infected plants in 1925, but negative results in 1926 and 1927. Martin has proved negative all three seasons. The material was originally collected many years ago in Missouri and has been constantly used in my experiments with *Tilletia laevis*.

Race IV is based on collection no. 2. It is able to infect Kanred and Turkey (717) to some extent. It is essentially negative on Turkey (729), Hussar, Martin, and Odessa. This material was obtained by Dr. J. A. Faris near Albany, New York. Collections nos. 3 and 4 have behaved in a very similar manner.

The remaining collections, results of which are summarized in table 1, are more or less similar to certain ones which are clearly differentiated. It may be that some of them are identical but, on the other hand, it may be possible to differentiate some of them, at least when further experiments have been carried out.

Tilletia tritici. Six races of this species seem to be clearly differentiated.

Race I is based on collection no. 2, which shows a marked capacity for infecting Hussar, Martin, Odessa, Kanred, and Turkey. High percentages of infection on these varieties were obtained. Although Turkey (717) gave negative results in 1927, there were infected in 1925 17 plants out of 29 (58.6 percent) and in 1926, 11 out of 16 (68.7 percent). The material was originally secured from Dr. F. Bubák. Three additional collections (nos. 3, 15, and 16) secured from Dr. L. Hecke, appear to be identical with collection no. 2. It is interesting to note, however, that another collection (no. 17) obtained from Dr. Hecke, gave negative results on these varieties.

Race II is based on collection no. 4. It attacks Kanred, Odessa, Turkey, and Hussar, but seems unable to go over onto Martin to any extent. In

1926 negative results were also obtained with this variety, 24 plants being grown; in 1925, however, 2 plants out of 9 (22.2 percent) were infected. This material was originally collected by Dr. J. A. Faris at West Seneca, New York.

Race III is based on collection no. 1. It attacks Kanred and Turkey (717) but is practically negative on Turkey (729), Hussar, Martin, and Odessa. The collection was originally obtained from Dr. Fred N. Briggs, California. Collection no. 6, obtained from Prof. G. L. Zundel, Washington; and no. 8 obtained from Dr. H. P. Barss, Corvallis, Oregon, appear to be very similar.

Race IV is based on collection no. 5. It produces a severe infection of Kanred and Turkey (717) and occasionally infects Odessa and Hussar. Martin, however, appears to be entirely resistant. The material was originally collected by Dr. Faris at Seneca, New York.

Race V is based on collection no. 10. It attacks Kanred and Turkey (717) and rarely Hussar, but appears unable to infect Turkey (729) and Martin.

Race VI is based on four collections (nos. 9, 14, 18, and 19) which were secured from England and one (no. 20) from Wales, through Dr. G. H. Pethybridge. All of these have proved to be negative on Hussar, Martin, and Odessa, and nearly so on Kanred and Turkey, the data for 1925 and 1926 being in close correspondence. It is interesting to note that Miss Sampson (11) has found several of these varieties resistant in her experiments with this smut in Wales.

Several of the remaining collections appear to be more or less identical. However, additional experiments with other varieties of wheat may serve to differentiate them.

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STUDIES ON THE GROWTH OF ROOT HAIRS IN SOLUTIONS VI. STRUCTURAL RESPONSES TO TOXIC pH AND MOLAR CONCENTRATIONS OF CALCIUM CHLORID¹

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In the preceding paper (V) of this series, data were presented in mathematical form as to certain features of roots and root hairs in solutions of calcium chlorid and calcium hydroxid, varying in molar and hydrogen-ion concentrations. These features included the length, diameter, and spacing of roots or root hairs or their parts. There are, however, certain other morphotic features which do not lend themselves readily to mathematical treatment. These will be described in this paper. The typical form of the root hair of *Georgia collards* and of most other roots is cylindrical, of uniform diameter, with a dome-shaped tip, straight, unbranched, and directed at right angles from the longitudinal axis of the root. This is the form which is characteristic of aquatic root hairs, that is, those arising and growing in an aqueous medium, in all concentrations except those which are very close to the acid or the alkaline limit of the hydrogen-ion range, or which are very close to the maximum molar concentration. The amphibious root hairs, that is, those which arise in air but continue to grow after immersion, show some of these abnormalities of form in all concentrations except those supporting the optimum growth rate. Others of the abnormalities are characteristic of the more toxic solutions only, so that they constitute more or less of a criterion of toxicity. In fact, although these form changes cannot be readily measured, they constitute the most conspicuous reaction of the root to changes in solution. The root proper also undergoes some modifications of form in very acid and very alkaline solutions, which will be likewise described below.

The morphotic response prevalent in most solutions is that which I have designated as *swelling*; that is, a temporary enlargement of the hair in diameter with a subsequent resumption of growth at approximately the original diameter. A series of stages in its development is shown in figure

¹ This paper is the last of a series of six appearing in successive issues of the Journal.

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1 *a-e*, Plate VII. The amphibious root hairs all develop these swellings immediately after immersion in all solutions except the optimum. Apparently they represent a period of adjustment of the root to the solution, after which the original form is resumed. Inasmuch as the root hairs are of a graded series of lengths at the time of immersion, these swellings appear several hours after immersion in a graded series of positions on these root hairs; and they thus constitute a distinct aid in determining the location of the youngest root hair at the time of immersion, the lengths of the various zones of the root during the growth-period in the solution, and the subsequent growth of the amphibious root hairs after immersion.

In interpreting the occurrence of root hairs of different diameters in different solutions, it was suggested in the preceding paper (V) that this might be due to a difference in the area of growth at the tip of the root hair. Confirmation of such an interpretation is seen in this phenomenon of swelling. It appears that the effect of immersion is to cause the deposition of cell-wall material over a wider area at the tip of the root hair, and hence to form a wider hair, in some cases very wide (fig. 5). These swellings are more common in weak salt solutions, inasmuch as there is a tendency for other form changes to replace them at high salt concentrations.

Schwarz (31) reported similar swollen root hairs in a 1-percent nutrient solution, and upon transfer from damp air to tap water. Sokolowa (33) likewise described and figured them, and interpreted the change in diameter of root hairs as a response to the oxygen content of the medium. Jeffs (13) noted a slight swelling of root hairs as a response to temperature changes.

The *capitate* form of root hair is apparently a modification of the swollen type, in which the growth at an increased diameter is followed by cessation of growth entirely, instead of a resumption of the original diameter. This is found to occur in high salt concentrations, and in very acid solutions of low salt content. It is figured in 0.120 CaCl_2 . It has been figured previously by Hill (10) and described by him as occurring in root hairs transferred from a strongly saline solution to fresh water.

The *inflated* type is apparently another modification of the swollen. In this case growth continues, but the original diameter is not resumed, the larger diameter being continued. It was found in all solutions with a molar concentration of 0.060 CaCl_2 , and in the more acid and alkaline solutions of lower concentrations. Above 0.060 M it increases in frequency (figs. 2, 11, 13, 14). It apparently indicates a higher degree of toxicity than the latter type, inasmuch as the increased area of cell-wall formation becomes permanent instead of temporary. On the plate it is figured in 0.060 M at pH 3.9 and a series of stages is represented from 0.100 M . Schwarz figured it (31, figs. 1, 2), and described it as occurring upon transfer from saturated air to 15-percent nutrient solution. It has also been figured by Hill (10) and Sokolowa (34).

Branching occurs quite commonly in weak solutions, and near the acid

and alkaline limits of all concentrations. It may take the form of a simple dichotomy, in which the two tips may (fig. 20) or may not (fig. 21) grow at the same rate. This bifurcation is evidently the result of the division of the growing region of the root-hair tip. It probably follows swelling, that is, a temporary arrest of growth and increase of area, though this is not always evident from the resultant form. In this event there is evidently a restriction of the growing area, after adaptation to the medium, to more than one locus instead of just one as in the case of swelling. Another type of branching is that in which the new growth is at right angles to the old growth, and at approximately the original diameter. This occurs in amphibious root hairs (figs. 9, 15) and in aquatic root hairs (figs. 6, 10). This form was also shown by Schwarz (31), Hill (10), and Sokolowa (34). It was found, together with the capitate, by Wortmann (190) and Zacharias (40) in sugar solutions. They described a thickened layer or cap, which is formed at the apex of the root hair, causing a cessation of growth. If the root is transferred to moist air. Zacharias found that a lateral branch would be sent out near the tip, and he attributed the cessation of growth to plasmolysis. Reinhardt (26) found that in a weak sugar solution the growth of the root hair of *Lepidium* ceased and that lateral branches were produced. Stiehr (35) changed the direction of growth of root hairs by means of the electric current. Watson (188) found hairs on old roots of *Helianthus rigida* forking.

Still another structural modification is the *curved* (fig. 9). It is the result of a progressive migration of the growing area in one direction. It is frequently associated with the swollen or with the last type of branching described above. In one instance it was found to occur in many root hairs which had been growing all night in the dark and were exposed to weak red light in the morning, curvature occurring after they were thus illuminated. Such modifications of form as are described above were first noted and described by Persecke (182). Reed and Orr (184) have shown the effect of hydrogen-ion concentration upon structure in their work with bacteria.

Marked injury to amphibious root hairs was found in solutions of a pH of 3.9 and 3.4, and of 9.4 and 10.4. In some cases these hairs were collapsed, and in others burst. The former were more characteristic of high concentrations, and bursting was more common in solutions near the acid and alkaline limits of all concentrations. The longer the amphibious root hair, the greater the likelihood of its collapsing or bursting. Pfeffer (23) first reported the bursting of root hairs in solutions; and Zacharias (40) confirmed these findings. The latter found that the outer wall might burst while the inner remained intact, and that growth might be resumed. Stiehr (35) found that root hairs of *Phleum* burst in KCl between 0.2 and 0.5 percent, in NaNO₃ between 0.15 and 0.25 percent, and in 0.5 percent K₂SO₄. Popesco (183) found that when roots were placed in solutions of

various dyes, the older root hairs did not become stained. He interprets this as indicating that the root hair is not an absorbing organ; whereas it may mean that it is injured by the solution. Miss Addoms has been making a thorough study of the coagulation of the protoplasm of root hairs in acid solutions (133), in concentrated (0.1 *N*) salt solutions, and in ultra-violet light (179). Weir (189) has recently studied similar phenomena in non-living colloids as caused by acids.

In our studies certain anatomical modifications of the root proper were also observed. They consisted of curvature, enlargement, constriction, and wrinkling or rupturing of the cortex. These modifications always occurred in regions of the root just distal to the region of root hairs. Subsequently root hairs emerge, usually on this modified part of the root. It seems therefore that this portion of the root is the most sensitive to variation in the milieu. It is the region of cell elongation, and has been known for a long time to be the chief motor region of the root in the case of tropic reactions.

There is in many cases a definite reaction of the root to the direction of flow of the solution. In some solutions this consists of a curvature upstream or downstream. In others there is a difference in the extent of the zone of root hairs on one side or the other, or there may be an entire absence of root hairs on the downstream or upstream side. Concentrations which show these reactions are only those which are very little different from those which are lethal for one reason or another. In alkaline solutions the curvature is commonly upstream and the root hairs are more numerous on the downstream side. In acid solutions the reverse is more likely to be the case, although exceptions are common. It would seem that such a reaction of the root to the solution flowing past it must be due to some effect which the root has upon the solution. It may be that the absorption of substances by the root on the upstream side reduces their concentration on the downstream side. This would result in a decreased toxic effect on the downstream side, which is indicated by the production of root hairs and the more rapid elongation of the root on that side. It is also probable that the liberation of carbon dioxide by the root may antagonize, or in some instances accelerate, the toxic effect of the solution. Whether it is proper to term this curvature a rheotropism or a traumatropism is not entirely clear from the data thus far presented. Hansteen-Cranner (9) noted similar root curvatures in solutions of metallic salts which were not flowing.

Enlargement of the region of cell elongation to a greater diameter than normal occurred in nearly all solutions above 0.100 *M* CaCl_2 . It is also occasionally found in very alkaline solutions of lower concentrations. It seemed to involve a thickening not only of the cortex but of the stelar tissue as well (Pl. IX).

Constriction of this region of cell elongation occurred, on the other hand,

in acid solutions. The constriction was throughout a length of from $\frac{1}{2}$ to 1 mm., and occurred at about 1 mm. from the root tip. Hansteen-Cranner (9) pointed out that the effect of toxic agents upon roots was first evident in the region of cell enlargement. He found that if roots were immersed in salts of the heavy metals, this region thickens, swells, and becomes gelatinous and glassy, secreting a slime. When transferred to calcium solutions the tissues were regenerated from outside in. In our study with calcium solutions the enlargement seemed to be a growth process, and not simply an hypertrophy. Neither was a slimy surface observed.

Rupturing of the cortex was found in alkaline solutions very close to the alkaline limit, as figured in Plate VIII. This was accompanied by, or perhaps resulted from, the curvature of the root first in one direction and then in the other. It appears as if the bundle grew but the cortex did not. Almost invariably the rupture occurred on the upstream side, where maximum injury might be anticipated on the basis of the interpretation of curvature given above. No reference has been found in the literature to the rupturing of roots in alkaline media. Sakamura (185) reports that the cells of *Gonium* separate in the chlorids of alkalis and alkaline earths except calcium chlorid, which antagonizes this macerating effect. He attributes the maceration to the dissolution of the pectic envelope. Schweizer (186), however, has pointed out some toxic effects of calcium upon radishes. It would appear that the rupturing which we have observed is due not so much to a macerating effect of the solution as to the growth of the central cylinder with a cessation of growth of the cortex.

Wrinkling of the cortex took place in acid solutions very close to the acid limit (Pl. VIII). This seemed to be the result of growth of the cortex without growth of the central cylinder. The three outer layers of cells are observed to pull away from the interior tissues at intermittent loci along the root. The rapidity of this change is indicated (figs. 1, 2) between 2 P.M. and 4:30 P.M., showing clearly that it is a growth reaction, and not due to sudden alteration of the cell-wall material. As in the case of the rupturing noted above, this seems to be the first description of such a reaction of roots to extreme hydrogen-ion concentrations. There is a wrinkling of the cortex in the contraction of roots, which is a normal process occurring in older parts considerably above the region of root hairs. It has not been found to be related in any way to the medium. Miss Church (181) has attributed it to the activity of the parenchyma between the bundle and the corky surface layers. Thoday (187) has recently studied it in *Oxalis*, and thinks it is due to the shrinkage of the parenchyma cells of the cortex. Whether the wrinkling which we find in response to hydrogen-ion concentration is due to a cessation of growth in the bundle or to an acceleration of growth in the cortex of the region of cell elongation, has not yet been determined.

In high concentrations of calcium chlorid there develops a definite zonation of the root (Pl. IX). These zones are, respectively: the root cap; the hairless root tip; the zone of aquatic root hairs; the interzone; the zone of amphibious root hairs. The last-named zone may be subdivided, due to the fact that the amphibious root hairs react differently to these high concentrations according to their ages, that is, their length at the time of immersion. In 0.105 *M* CaCl_2 there are four sub-zones of this zone of amphibious root hairs. Next to the interzone these root hairs are of normal form; next there is a sub-zone of inflated root hairs; then a sub-zone of hairs which have not grown since immersion though they appear normal in form; finally, the longest root hairs are collapsed. These four sub-zones may be referred to as the *normal*, *inflated*, *stagnant*, and *collapsed*. At a concentration of 0.080 *M* CaCl_2 there is no interzone and no inflated nor stagnant root hairs, that is, all of the amphibious root hairs are growing normally except the very longest, which may be collapsed upon immersion. With increase of concentration of the neutral solution there appears first the interzone, then the inflated root hairs, and finally the stagnant. Above 0.105 *M* the number of collapsed root hairs is greater with increasing concentration, and the number of normal less. The normal amphibious root hairs are absent entirely at 0.010 *M* and above. In 0.120 *M* the number of inflated root hairs has decreased, and the number of collapsed hairs increased correspondingly. Meanwhile the length of the zone of aquatic root hairs has decreased also. If this concentration is rendered acid, that is, pH 4.4, the inflated amphibious root hairs do not appear, the youngest root hairs being now stagnant and the aquatic root hairs becoming inflated. At pH 3.4 no aquatic root hairs are produced at all. There is thus with increasing concentration of the salt, and then of the hydrogen ion, a progressive shifting of the zones and a gradual disappearance of them. This recalls the work of Child (180) on axial gradients.

SUMMARY VI

54. Root hairs which have begun to develop in air, when placed in even slightly toxic calcium chlorid solutions, become modified in form by swelling, inflation, branching, or curvature. The more toxic the concentration the more extreme the modification. In very toxic solutions they burst and collapse.

55. Root hairs newly formed in the solution may show abnormally large diameter and irregular form in concentrations which are almost lethal.

56. The modifications in form of root hairs in different solutions is interpreted as due to an effect upon the extent of the area of cell-wall deposition at the tip. The literature upon structural modifications of root hairs is reviewed.

57. The region of cell elongation of the root commonly becomes of greater diameter in solutions of high salt concentration, or of low hydrogen-

ion concentration. In acid solutions it is frequently constricted in this region. In very acid solutions of median salt concentration, the cortex may become wrinkled and pull away locally from the bundle. In alkaline solutions the bundle continues to elongate, rupturing the cortex. These modifications of the root are largely attributable to a differential effect of the solution on the stele and on the cortex, respectively.

58. At high concentrations of salt the root shows a marked zonation, with a shift in the zones and an elimination of the zone of aquatic root hairs and of normal amphibious ones as the concentration is increased or made more acid.

59. In very toxic concentrations the root shows a curvature with respect to the direction of flow of the solution, and also a difference in the production of root hairs on the upstream and downstream sides, respectively.

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EXPLANATION OF PLATES

PLATE VII

Abnormal forms of root hairs in calcium chlorid solutions

FIG. 1, a-e. Development of a swollen amphibious root hair in 0.004 M at pH 7.9. Intervals: 2 hours.

FIG. 2, a-h. Development of an inflated amphibious root hair in 0.100 M at pH 7.9. Intervals: 1 hour.

FIG. 3, a, b. Development of a bulged aquatic root hair in 0.120 M at pH 5.9, showing migration of nucleus. Interval: 20 minutes.

- FIG. 4. Pyriform or spatulate amphibious root hair in 0.120 *M* at pH 5.9.
 FIG. 5. Globose or flask-shaped amphibious root hair in 0.060 *M* at pH 3.9.
 FIG. 6. Forked or branched aquatic root hair in 0.120 *M* at pH 6.9.
 FIG. 7. Forked and inflated amphibious root hair in 0.060 *M* at pH 3.9.
 FIG. 8. Crooked and inflated amphibious root hair in 0.120 *M* at pH 6.9.
 FIG. 9. Curved or sickle-shaped amphibious root hair in 0.100 *M* at pH 7.9.
 FIG. 10. Forked or branched aquatic root hair in 0.008 at pH 11.9.
 FIG. 11. Inflated aquatic root hair in 0.120 *M* at pH 7.4.
 FIG. 12. Swollen, inflated, curved, and forked amphibious root hair in 0.112 *M* at pH 7.9.
 FIG. 13. Inflated aquatic root hair in 0.120 *M* at pH 6.9.
 FIG. 14. Inflated aquatic root hair in 0.120 *M* at pH 7.4.
 FIG. 15. Forked amphibious root hair in 0.120 *M* at pH 7.4.
 FIG. 16. Swollen, forked, and sickle-shaped amphibious root hair in 0.120 *M* at pH 7.4.
 FIG. 17. Swollen and forked amphibious root hair in 0.120 *M* at pH 7.4.
 FIG. 18. Swollen, kinked, and crooked amphibious root hairs in 0.120 *M* at pH 7.9.
 FIG. 19. Capitate amphibious root hair in 0.120 *M* at pH 6.9.
 FIG. 20. Forked or branched amphibious root hair in 0.008 *M* at pH 8.9, both branches growing equally well.
 FIG. 21. Forked or branched amphibious root hair in 0.008 *M* at pH 8.9, one branch having ceased growing.
 FIG. 22. Kinked amphibious root hair in 0.112 *M* at pH 8.2.

PLATE VIII

Reactions of roots to extreme hydrogen-ion concentrations

FIG. 1. Root in 0.060 *M* calcium chlorid at pH 3.9, showing wrinkling of cortex in region of root-hair initiation.

FIG. 2. A portion of the same root two and one-half hours later, showing the rate of wrinkling due to more rapid growth of the cortex. This portion is the same as that shown beside it in figure 1.

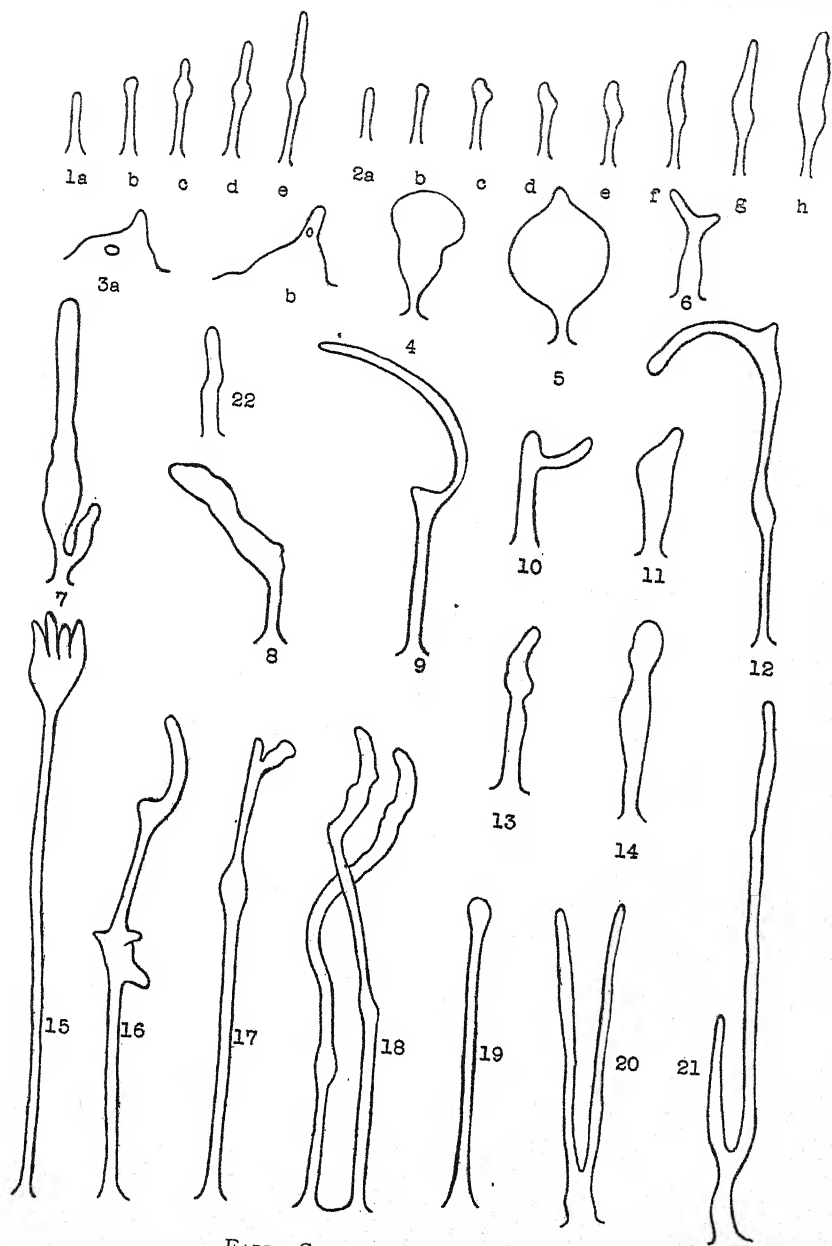
FIG. 3. A root in 0.028 *M* calcium chlorid at pH 10.4, showing marked curvature toward the direction from which the solution is coming, and the formation of root hairs only on the side away from the current.

FIG. 4. The same root seven hours earlier, showing the rupturing of the cortex on the side toward the current. These ruptures are for the most part closed by the curvature shown in figure 3.

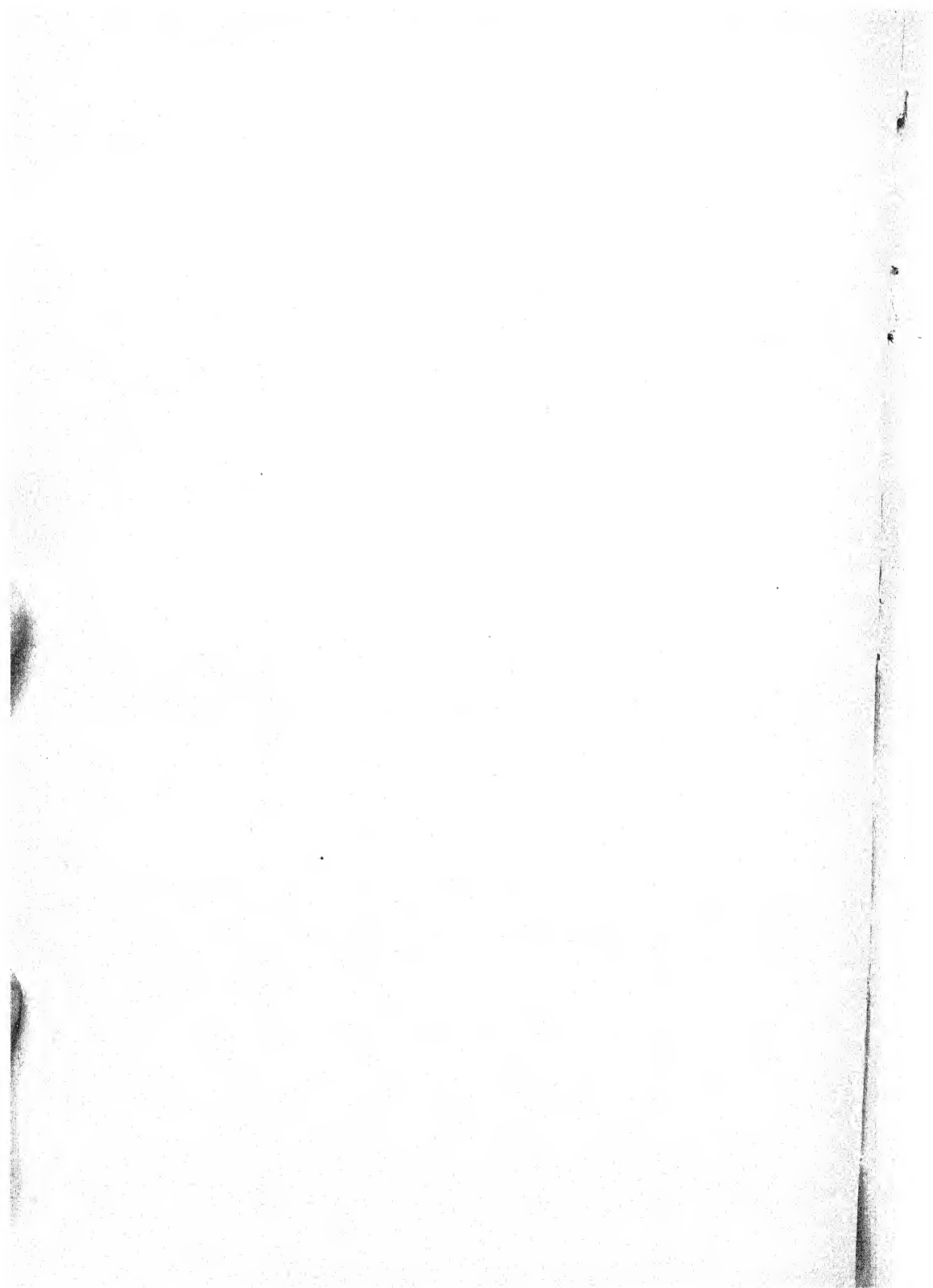
FIG. 5. A root in 0.020 *M* calcium chlorid solution at pH 9.9, showing curvature and rupture of the cortex entirely through to the endodermis.

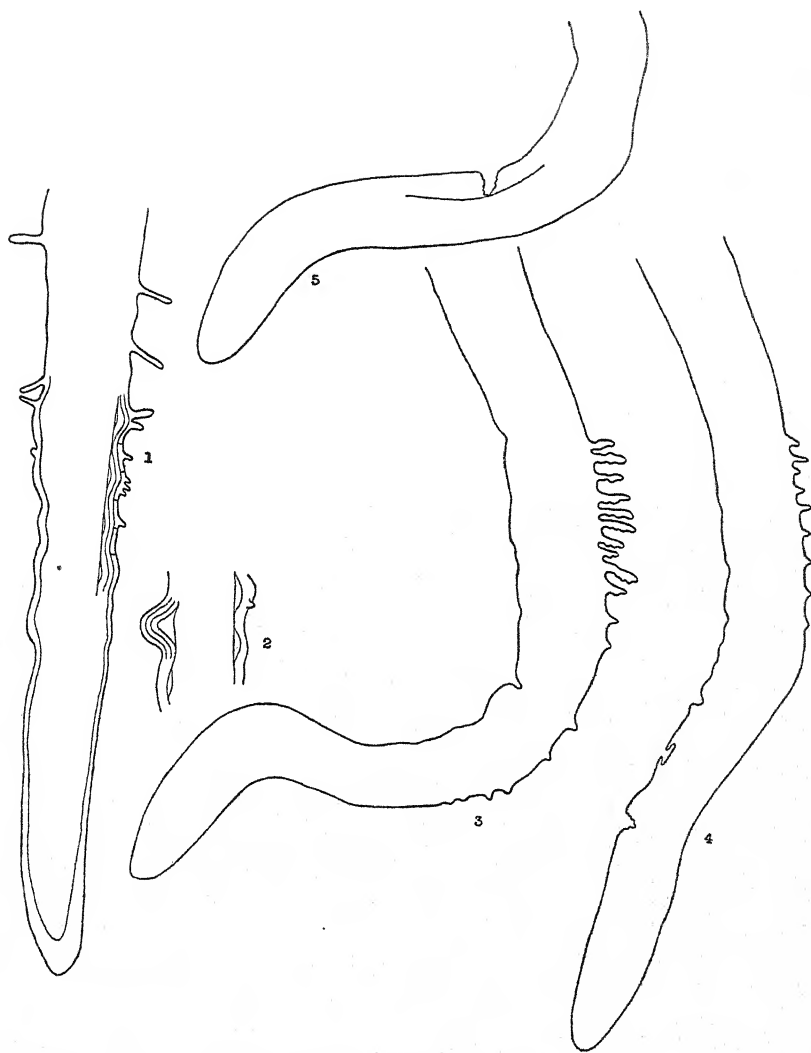
PLATE IX

Zonation and modification of root hairs at high concentrations of calcium and hydrogen ions. The meanings of the symbols are as follows: *a*, amphibious; *c*, collapsed; *g*, no growth; *f*, inflated; *i*, interzone; *z*, zone of aquatic root hairs; *t*, hairless tip; *n*, normal root hairs. The drawing shows the zonation in 0.110 *M* at pH 7.9. The zonation in this and the other concentrations is indicated by the spaces below, showing that with increased calcium and hydrogen-ion concentration, there is an extension of the zone of collapsed root hairs, first at the expense of the normal, then of the inflated, and finally of the region of no growth. Likewise there is a progressive extension of the interzone, with a diminution of the length of the zone of aquatic root hairs and a shortening of the hairless tip, until the interzone and the hairless tip become joined together by the obliteration of the zone of aquatic root hairs entirely. Just before this is obliterated, however, all of the aquatic root hairs become inflated.



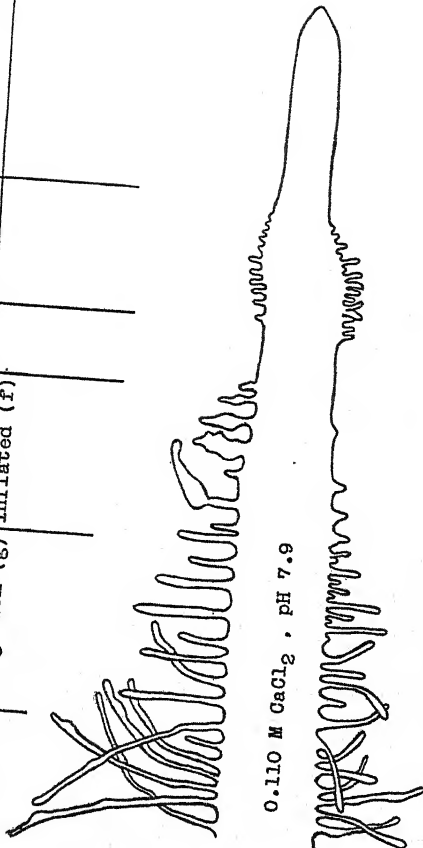
FARR: GROWTH OF ROOT HAIRS





FARR: GROWTH OF ROOT HAIRS

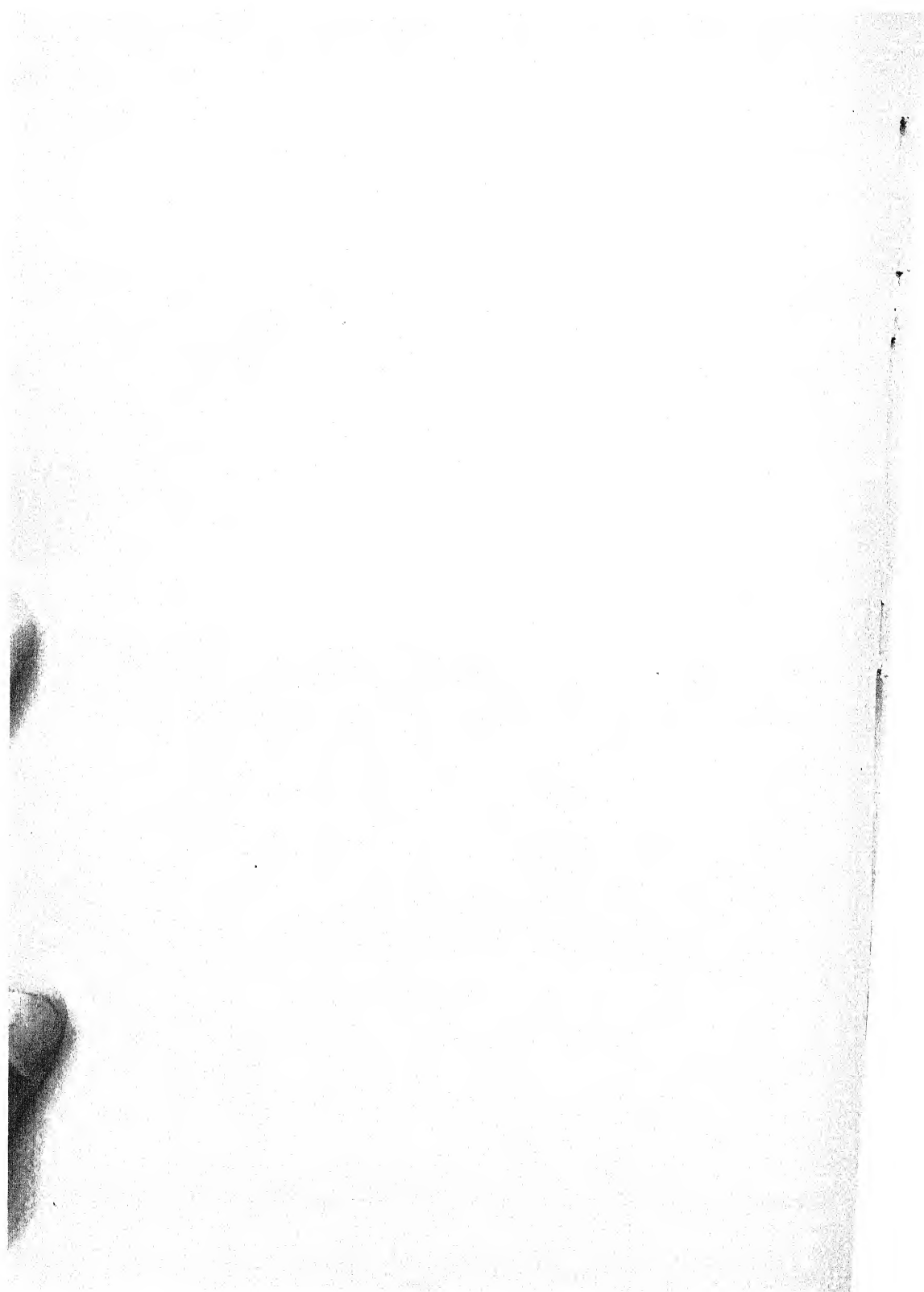
Amphibious root hairs (a)			
collapsed (c)	no growth (g)	inflated (f)	
		(i)	(z)
			(t)



0.110 M CaCl_2 . pH 7.9

conc.	pH										
0.080	7.9	ac	an				zn				t
0.100	7.9	ac	af	an			i	zn	t		
0.105	7.9	ac	ag	af	an		i	zn	t		
0.110	7.9	ac	ac	ag	af	an	i	zn	t		
0.120	7.9		ac	ag	af	an	i	zn	t		
0.120	4.4		ac		ag	af	i	zn	t		
0.120	3.4		ac			ag	i	zf	t		
			ac				t				

FARR: GROWTH OF ROOT HAIRS



GERMINATION OF THE SPORES OF *PELLIA EPIPHYLLA* AND *P. NEESIANA*

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Observations on the germination of *Pellia* spores were first recorded by Hofmeister (1851) in his classical "Vergleichende Untersuchungen." He observed the succession of the early divisions in sporelings of *Pellia epiphylla* Corda. Mueller (1866) studied *P. Fabroniana* (*P. calycina* [Tayl.] Nees). His description for *P. epiphylla* agreed in general with that of Hofmeister, though he added figures showing several exceptions to the usual method of development. Leitgeb (1877) also observed the germination of spores of *Pellia* and agreed with Hofmeister as to conditions in *P. epiphylla*. He extended his observations to *P. Fabroniana*, discovering the essential features of the development and remarking upon the differences existing between the two species in this respect. Recently Showalter (1925) described the germination of the spores of *P. Fabroniana* and attempted to determine the origin of the apical cell.

In stained sections of sporogones of *P. epiphylla* Corda I have been able to trace the development of the young gametophyte from the undivided spore to the seven- and nine-celled stage at which the so-called "spores" are freed. Studies of the further development were made on living material grown in water and on agar, and on young plants fixed and stained and mounted in glycerin.

The material for the study of *P. epiphylla* was taken from greenhouse cultures made from plants collected in the vicinity of Brussels, Belgium. The material of *P. Neesiana* (Gottsche) Limpr. was kindly furnished by Doctor A. M. Showalter from his greenhouse cultures. The original plants from which these latter cultures were made were collected in the Black Forest in Baden (Germany).

PELLIA EPIPHYLLA

Hofmeister and Leitgeb reported that the sporelings of *P. epiphylla* when shed usually consist of four cells, but in all the mature sporogones which I examined the majority of the sporelings contained seven or nine cells. Miss Greenwood (1911) figures two sporelings containing five and six cells, respectively.

The one-celled spore just previous to the first division measures, approximately, from 68 to 76 microns in length and from 35 to 42 microns in breadth. There is some growth within the sporogone, and measurements

of four-celled sporelings showed a length of about 85 microns and a breadth of about 51 microns.

At the seven-celled stage just previous to the time of shedding, the length of the sporelings was found to be about the same (85 microns) and the breadth had increased to about 60 microns, so that in the somewhat older sporelings the increase in breadth is greater than that in length.

In the young sporelings the cytoplasm is quite dense and contains numerous plastids. In the older ones the vacuoles are much larger, the cytoplasm consequently appearing less dense and the plastids more scattered. The protoplasm of the basal cell is always considerably less dense than that of the other cells of the sporeling. This is often noticeable from the time this cell is formed, *i.e.*, at the four-celled stage.

Probably, as Showalter (1925) has reported for *P. Fabroniana*, the basal cell of any given sporeling of a quartet is in contact with the basal cells of the three others (Pl. X, fig. 10). This point could not usually be determined with certainty because of the early separation of the sporelings from one another, and the shifting of their position in the sporogones in consequence of growth.

Figures 1-10 (Pl. X) represent the development of the young gametophyte to the time it is freed from the sporogone. The first division is transverse (Pl. X, fig. 1). The two resulting daughter cells are divided simultaneously (Pl. X, fig. 2) in transverse planes (Pl. X, fig. 3). The sporeling is now a row of four cells; the two end cells taper and the two central ones have approximately the form of flattened cylinders, though they taper very slightly in the direction of the end cells. Hofmeister described this stage as consisting of "four cells arranged in a single row, two of which are disc-shaped and two hemispherical." From this point on, as already stated, the apical and basal ends of the young gametophyte can often be recognized, the basal cell containing less dense cytoplasm and fewer plastids.

The next (third) division is longitudinal and may occur simultaneously (Pl. X, fig. 4) in the two central cells; the resulting wall in each cell is approximately central and at right angles to the previous walls (Pl. X, fig. 5). These two latest walls may or may not be in the same plane. It was observed that the long axes of the spindles which preceded their formation are not always parallel. Figure 4 (Pl. X) shows two spindles whose axes are at right angles to each other, while in figure 17 (Pl. XI) the axes of the two spindles are parallel. There is no reason to think that the axes of the spindles change during mitosis, or that the cell wall is laid down in any plane other than one at right angles to the axis of the spindle. One would therefore expect that a sporeling such as is represented in figure 4 (Pl. X) would proceed to develop two longitudinal walls whose planes would be at right angles to each other, while a sporeling such as is shown in figure 17 (Pl. XI) would develop two longitudinal walls in the same plane (Pl. X, fig. 5).

In the sporeling shown in figure 6 (Pl. X) only one of the central cells has divided. In this case the following division would occur in the other central cell (Pl. X, fig. 5) or in the cell at the apical end (Pl. X, fig. 7).

A second longitudinal division often occurs in one of the central cells next to the basal end cell (Pl. X, figs. 9, 10). Figure 10 (Pl. X) represents the most advanced stage of development that I have seen while the sporelings were still enclosed in the sporogones.

At the time of shedding the sporeling usually consists of from six to nine cells (Pl. X, figs. 7-10): a basal cell, four or six central cells, and an apical end consisting of one or two cells.

Further stages in the development of the young gametophytes may be observed by allowing the spores to remain in tap water or by sowing them on agar in Petri dishes.

Observations were made upon the resistance of freshly shed sporelings to drying, and, as was to be expected on account of the very thin spore wall, this resistance was found to be slight. After a very short exposure (10 to 15 minutes) to the warm, dry air of the laboratory the sporelings collapse. If replaced immediately in water they soon become turgid again and continue their growth apparently unharmed. If they are allowed to remain on a dry slide for several hours or even for several days and are then replaced in water, some will still become turgid and grow. It was observed that almost no spores were capable of continuing their growth after having been left on dry slides in the laboratory for a period of one week or more. Obviously, the higher the temperature to which the young plants are exposed and the lower the relative humidity of the atmosphere, the shorter the time of exposure which will allow of recovery.

Within a few days after placing freshly shed sporelings in water or on agar, divisions occur in all the cells except the basal one; this, however, sometimes divides longitudinally (Pl. X, fig. 11). There are no further divisions at this end of the sporeling. A protrusion appears on the basal cell (Pl. X, fig. 13) and grows out to form the first rhizoid. The place of development of this rhizoid is probably variable. The second rhizoid may also arise from the basal cell or from one of those adjacent to it.

A number of oblique divisions occur in the apex, forming cells broader at the anterior than at the posterior end (Pl. X, figs. 12, 13). One of these probably continues to function as an apical cell. There is considerable variation in the number, form, and size of these cells in different sporelings. Forking of the thallus sometimes occurs very early in development and this may be due to the functioning of two of these anterior cells as apical cells. The beginning of such a method of development may have been in process in the sporeling represented in figure 14 (Pl. X). The transverse divisions in the apical part of the young thallus suggest that the two larger cells of the anterior transverse row might now function as apical cells. I have not been able to follow this process further.

PELLIA NEESIANA

The germination of the spores of *P. Neesiana* was studied in living material and stained preparations in the same manner as that described for *P. epiphylla*. The development of the two species differs in no way as far as I can determine, and those who consider *P. Neesiana* merely as a dioicous variety of *P. epiphylla* may see here further evidence in favor of their view.

A series of early stages in the development of the gametophytes is shown (Pl. XI, figs. 15-26). Figure 15 (Pl. XI) is the four-celled stage corresponding to figure 3 (Pl. X) of the *P. epiphylla* series. Figure 16 (Pl. XI) shows the result of the following division (*cf.* Pl. X, fig. 6). Figure 17 (Pl. XI) shows the third division occurring simultaneously in both central cells. The axes of the two spindles of this division may be at right angles to each other as shown in figure 4 (Pl. X). Figure 18 (Pl. XI) represents a succeeding stage in which a longitudinal division has occurred in the two central cells and in the cell at the apical end.

The figures following were drawn from living material growing on agar. Figure 19 (Pl. XI) represents a sporeling which has grown considerably and in whose central group of cells several longitudinal divisions have taken place. Figure 20 (Pl. XI) shows the beginning of the simultaneous development of two rhizoids from the basal cell. Figures 21 to 25 (Pl. XI) show stages in further development, including the formation of a group of wedge-shaped cells in the apical region, one of which (or two if forking occurs immediately) functions as an apical cell. Showalter (1925) also figures such a cell and considers that it functions as an apical cell. In young living plants at more advanced stages an apical cell is frequently discernible and is probably always present as in the mature gametophytes.

A case of the development of a thallus from the side of a sporeling is shown in figure 26 (Pl. XI). Mueller (1866) figured a similar case.

DISCUSSION

The results of spore-germination studies in the Hepaticae permit of little generalization. There is so much variation among the different genera of any group in this respect that a single type of spore-germination may not be considered a characteristic of a group. In the acrogynous Jungermanniales there is considerable variation, germination taking place either by the immediate formation of a cell mass or by the growth of a protonema, either simple or branched. Among the Anacrogynae, *Pellia* stands out from the other members of the group because of the fact that the spores form a multicellular body while retained within the capsule. While a cell mass is also formed in the other Anacrogynae, it is produced after the spores are freed from the capsule, and the method of its development varies among the different forms. It is interesting to note that *P. Fabroniana*, a species closely related to the two here studied, reveals a

distinct embryogeny (Leitgeb 1877, Showalter 1925). It may be noted also that environmental conditions, such as higher or lower temperatures, different substrata, etc., may modify materially the course of development.

SUMMARY

In *Pellia epiphylla* and *P. Neesiana* the first division of the spore is transverse. The second division is also transverse and occurs simultaneously in both cells, forming a row of four cells. The probable relation of the four sporelings of a quartet to one another is that the basal cells of all are in contact. The basal cell does not usually divide, though there may be a single longitudinal division. Longitudinal divisions now occur in the other cells, usually one in the cell at the apical end, several in the central cells.

The sporelings show very slight capacity to resist desiccation.

After the sporelings become free there is less regularity in the divisions. In general, more longitudinal divisions occur in the central region and more oblique ones in the apical region, the latter divisions forming cells broader at the anterior than at the posterior end. One of these probably functions as an apical cell.

The results here recorded were obtained during the writer's tenure of a Commission for Relief in Belgium Educational Foundation Fellowship and of a National Research Fellowship in the Biological Sciences.

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DESCRIPTION OF PLATES

The drawings were made with the aid of a camera lucida. The figures in Plate X are reproduced at a magnification of 335 and those of Plate XI at a magnification of 145.

PLATE X

Pellia epiphylla

- FIG. 1. Equatorial plate stage of first division and first wall.
FIG. 2. Simultaneous metaphases of second division.
FIG. 3. Second division completed, showing three transverse walls.
FIG. 4. Metaphase of third divisions, one in polar, one in side view.

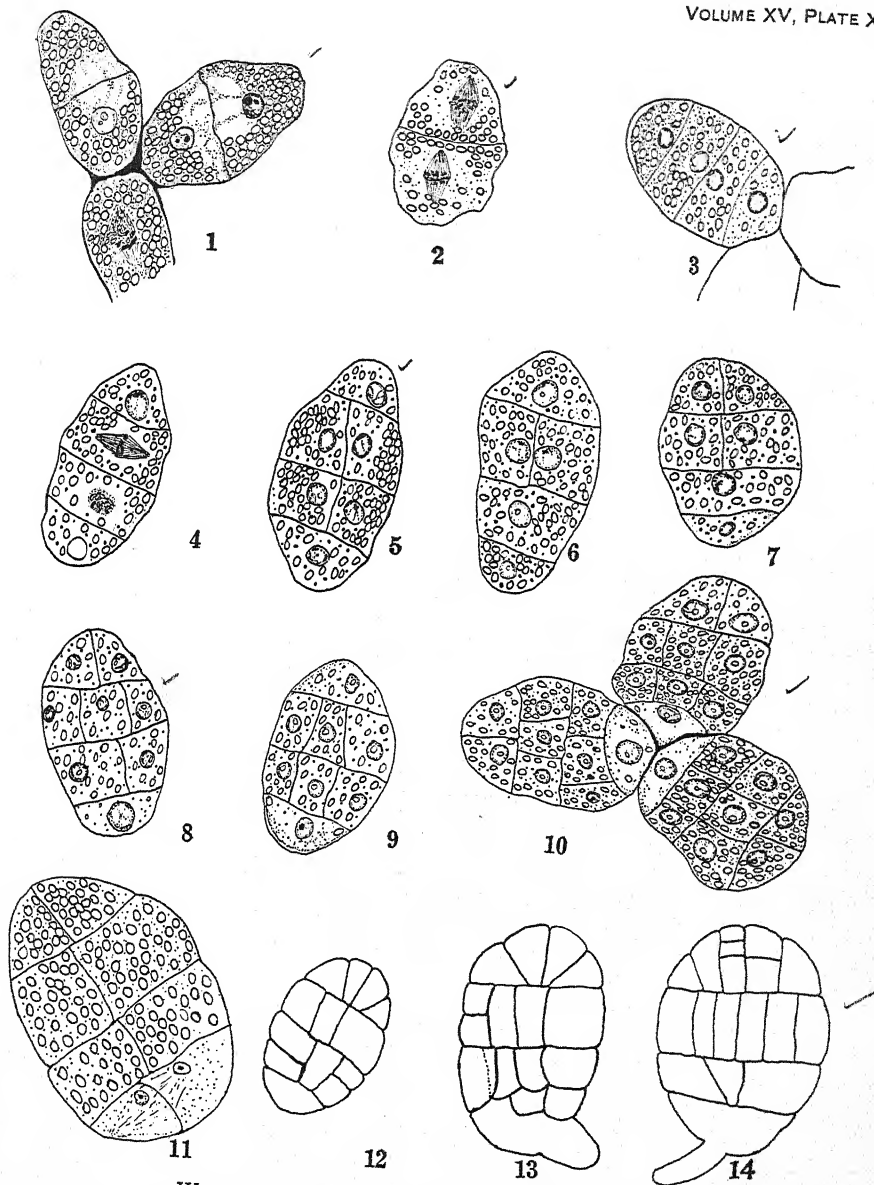
- FIG. 5. Third divisions completed.
FIG. 6. Result of division of only one of the central cells.
FIG. 7. Result of longitudinal division of one of the central cells and of the cell at the apical end.
FIG. 8. Third longitudinal wall formed in the central group of cells.
FIG. 9. Fourth longitudinal wall formed in the central group of cells. Longitudinal division has not yet occurred in the cell at the apical end.
FIG. 10. Three sporelings of a quartet, showing the relative positions of basal cells.
FIG. 11. Sporeling from water culture, showing longitudinal division in basal cell.
FIGS. 12-14. Semi-diagrammatic sketches from living material, showing further development of apical region and growth of rhizoid ($\times 220$).

PLATE XI

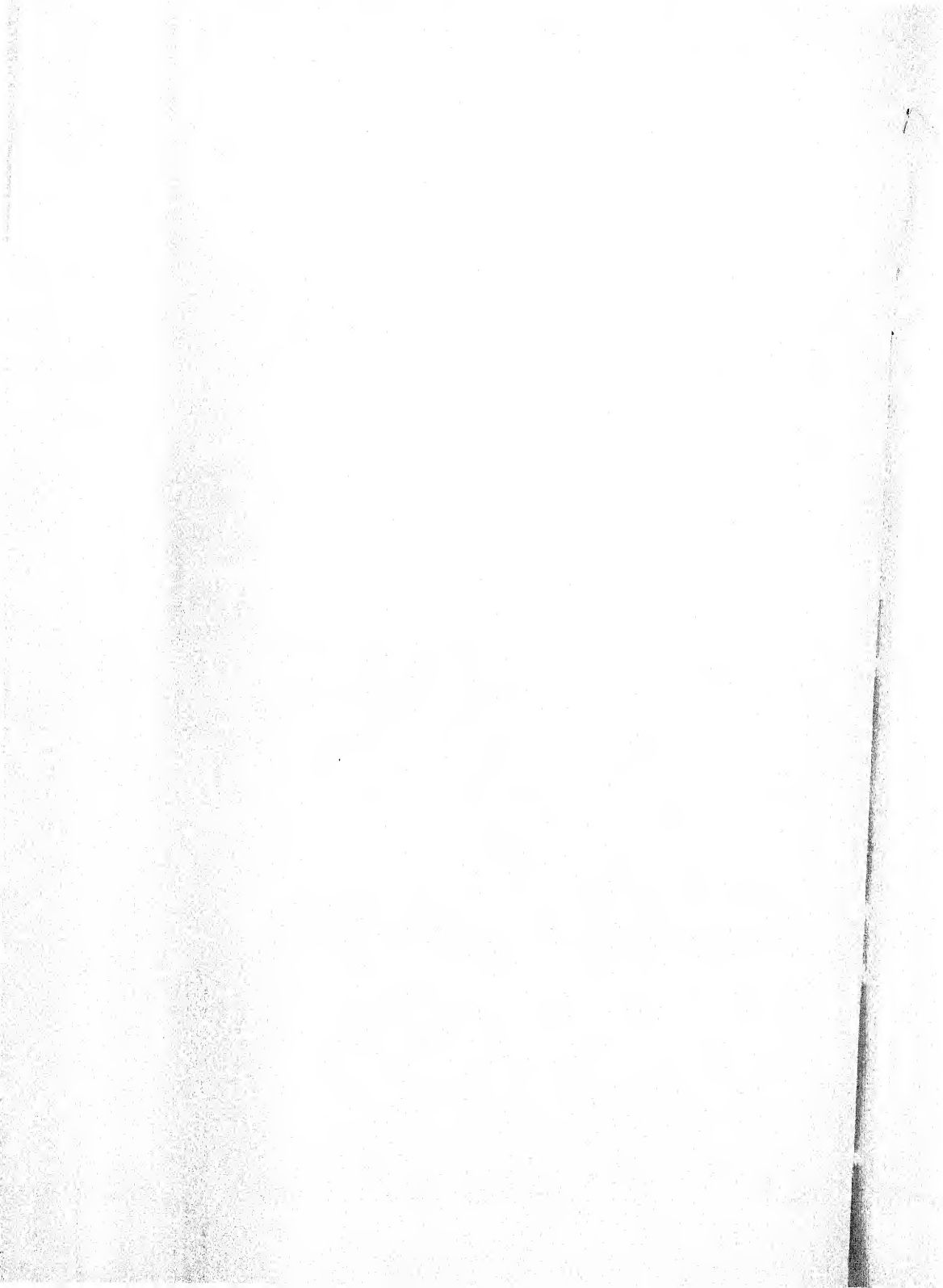
Pellia Neesiana

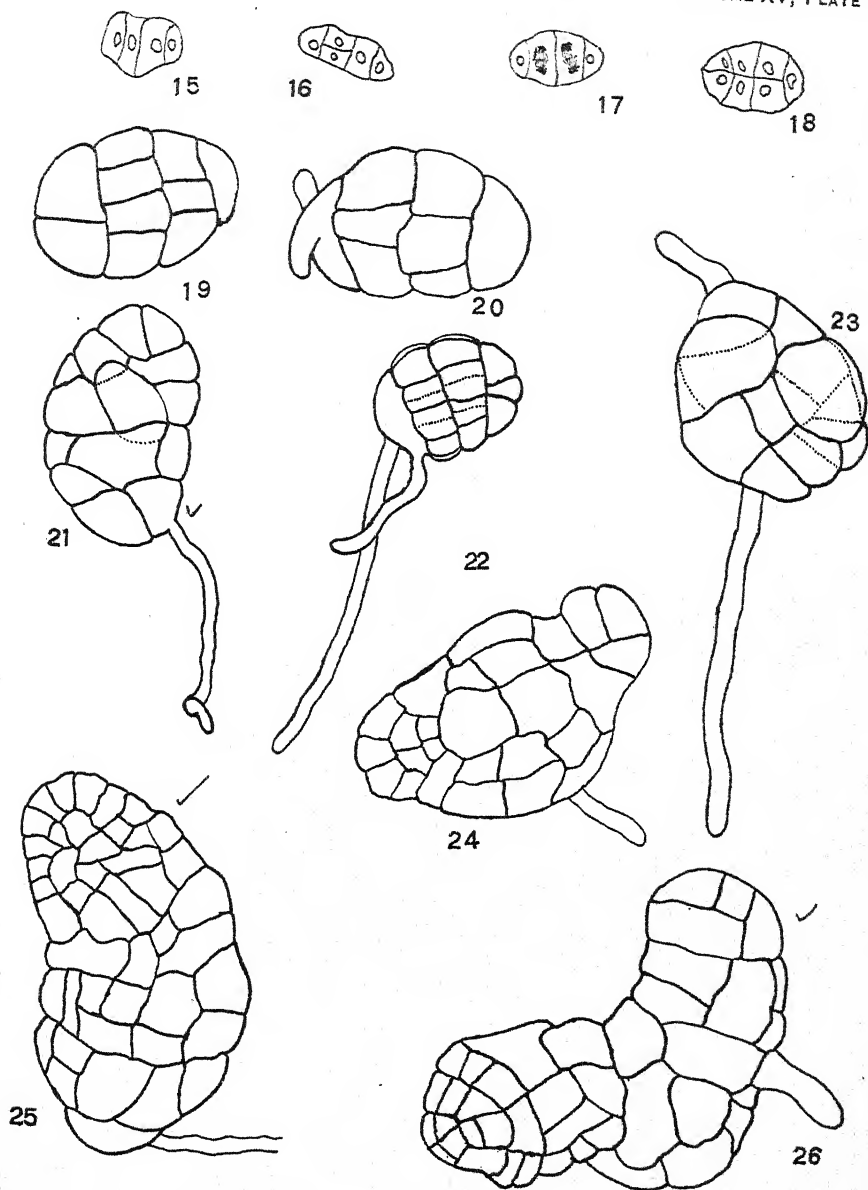
The more advanced stages are partly diagrammatic. Figures 15-18 drawn from sporelings contained within the sporogone. Figures 19-26 drawn from living material on agar.

- FIG. 15. Four-celled sporeling.
FIG. 16. Five-celled sporeling.
FIG. 17. Telophases of third division, showing formation of first longitudinal walls.
FIG. 18. Seven-celled sporeling.
FIG. 19. Later stage, after longitudinal divisions have occurred in central cells.
FIG. 20. Origin of rhizoids.
FIGS. 21-23. Probable origin of apical cell.
FIGS. 24-25. More advanced stages.
FIG. 26. Formation of thallus from side of sporeling.

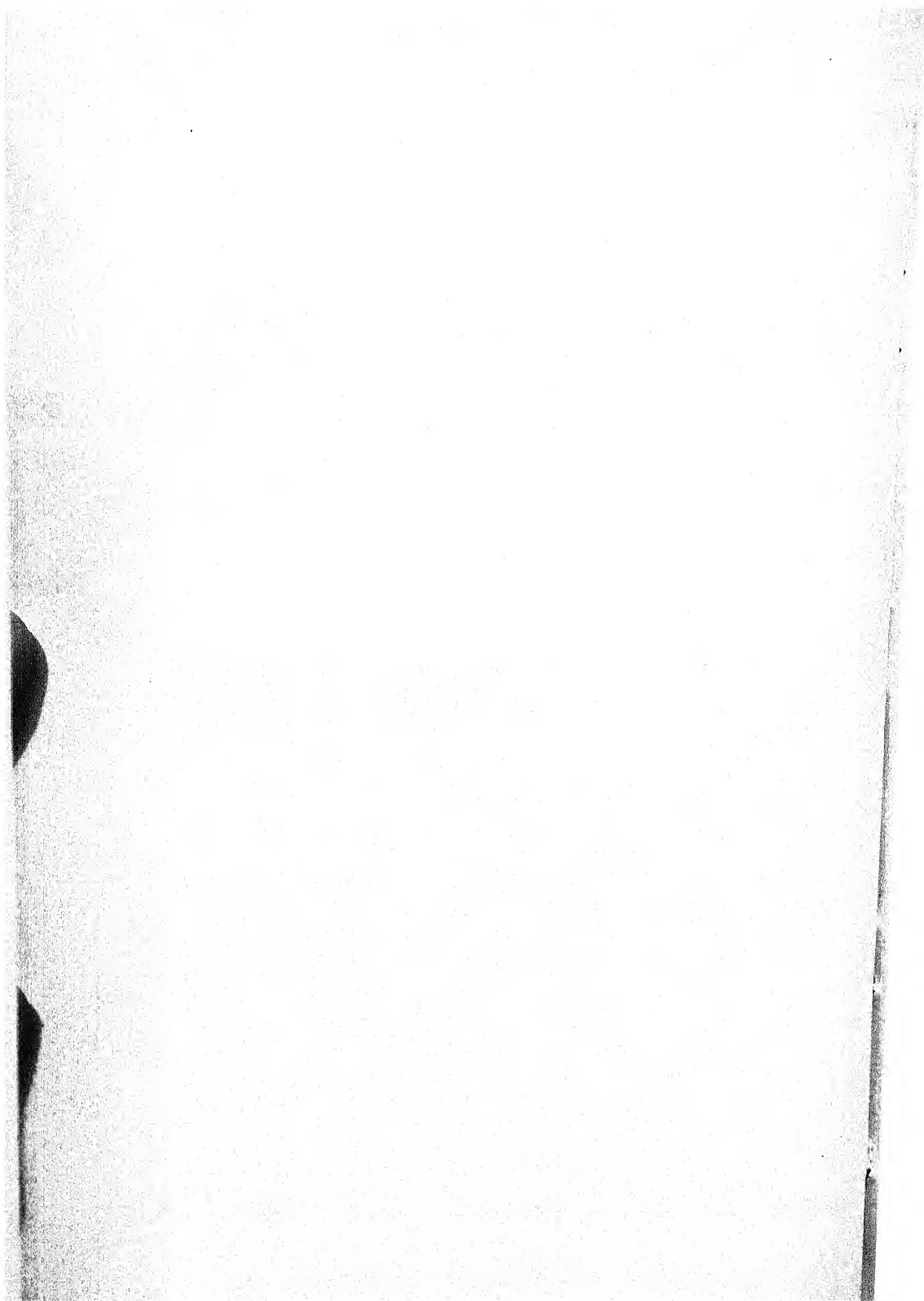


WOLFSON: GERMINATION OF PELLIA SPORES





WOLFSON: GERMINATION OF PELLIA SPORES



LIGHT INTENSITIES REQUIRED FOR GROWTH OF CONIFEROUS SEEDLINGS

C. G. BATES AND JACOB ROESER, JR.

(Received for publication July 21, 1927)

In an earlier communication¹ the senior author described the first of a series of experiments in growing seedlings of coniferous trees under artificial light for the primary purpose of establishing the extent of difference between species, if any exists, with respect to so-called "tolerance of shade" or ability to grow in weak light such as is sometimes found under the forest canopy.² Very marked differences between species have been reported frequently by foresters, but there has always been an element of doubt as to the part which keen competition for moisture, and other factors concomitant with greater or less shading, might play in the phenomena commonly recorded in the field. The very great significance of actual differences between species will be readily understood. They might play an important part in natural regeneration of any forest, in a succession of types, and in every plan made by the forester, based on cutting or any other treatment which affects the light supply. Broadly speaking we can not afford to give space in the forest to a species which is relatively inefficient in photosynthesis, unless it is producing a wood of exceptional technical value.

No attempt is made at this time to review the literature of the subject, which is voluminous and covers a multitude of viewpoints, beyond saying that direct experiments to establish the degree of photosynthetic activity of different species have been very few. Very little of a precise character has been learned about any of our American trees.³ Hence the present series of experiments, which centers about a group of four Rocky Mountain conifers which have been studied from a number of physiological angles.

METHODS

The methods followed in the first test and in the one whose results are here described were essentially similar, and very simple. In this latter test a circular table 7 feet in diameter was employed, carrying a layer of

¹ Bates, C. G. The relative light requirements of some coniferous seedlings. *Jour. Forestry* 23: 869-879. 1925.

² But as pointed out by Burns (*Vermont Agr. Exp. Sta. Bull.* 193, 1916; and *Bull.* 261, 1927) and others, light in the forest is often of high intensity for short periods, but very intermittent in character, making the problem much more complicated than it at first seems.

³ Except by Burns, G. P. Minimum light requirement referred to a definite standard. *Vermont Agr. Exp. Sta. Bull.* 235, 1923.

rich loamy soil 5 inches in depth. The seeds of each species were broadcasted over this area ⁴ in such manner that seedlings would develop at all distances from the center of the table and source of light. For sixty days sunlight was admitted and conditions conducive to prompt germination were maintained, after which the room was completely darkened and the only source of light was a 200-watt, tungsten-filament, blue-glass lamp suspended over the center of the table and 12 inches from its surface. This light was turned on for 10 hours a day, through a period of 9 months. There was thus supplied, immediately below the lamp, light with a total energy value about $1/8$ (13.08 percent) as great as that of sunlight and at the extreme edge $1/160$ as great, and each species had opportunity to grow in light of any intermediate intensity.

TABLE I. *Scale of Light Values by Coblentz Thermopyle* †

Distance From Center of Table (inches)	Light Intensity (percent)	Distance From Center of Table (inches)	Light Intensity (percent)
0	13.08	21	2.46
1	12.97	22	2.26
2	12.65	23	2.10
3	12.13	24	1.95
4	11.48	25	1.81
5	10.68	26	1.70
6	9.79	27	1.59
7	8.90	28	1.48
8	8.01	29	1.38
9	7.26	30	1.28
10	6.60	31	1.19
11	6.05	32	1.10
12	5.52	33	1.02
13	5.06	34	0.97
14	4.61	35	0.92
15	4.21	36	0.87
16	3.84	37	0.82
17	3.50	38	0.78
18	3.19	39	0.74
19	2.90	40	0.71
20	2.68		

† Computed to an elevation 1 inch above surface of soil and based on the mean noon galvanometer deflection of 40 units for the summer of 1926, clear days.

Both sunlight values (the standard here is mean maximum noon light for the summer of 1926) and those artificial values obtaining at different points on the table were measured with a Coblentz thermopyle and galvanometer (table 1). It is, of course, to be understood that with the above-described lamp there is yielded a light much richer in red rays than is sunlight, and almost wholly devoid of ultra-violet. What effect this may have on the absolute light requirements shown, it remains for later qualitative tests to

⁴ To avoid confusion between seedlings of similar appearance, the table was divided at this time into four quadrants and certain of the species were confined to a half or a quarter of the entire area.

TABLE 2. Data on the Conditions of Experiment Prior to Use of Artificial Light
Weights in milligrams

Species and Origin	Quad. in Which Sown	Seed Quality		Dry Weight of Seedlings at Close of Germination Period				Probable Error† in Average
		Average Weight of Seeds	Number Sown	Number Germinating in 60 Days	Number Measured	Highest Weight	Lowest Weight	Average Weight
<i>Pinus edulis</i> (unknown).....	All	315.2	175	172	7	155	71	104.7
" <i>Lambertiana</i> (Calif.).....	1	229.7	100	1*	3	67	50	59.0
" <i>flexilis</i> (Colo.).....	All	93.5	200	121	7	69	30	47.3
" <i>ponderosa</i> (Ariz.).....	1 & 2	34.9	500	312	9	50	32	39.0
" <i>ponderosa</i> (Colo.).....	3 & 4	40.9	500	468	7	41	12	26.0
" <i>Strobilus</i> (Minn.).....	2 & 3	12.85	500	49	5	10	6	8.0
" <i>monticola</i> (Idaho).....	4	14.92	250	4*	1	—	—	5.0
" <i>resinosa</i> (Minn.).....	1 & 4	7.94	500	311	14	16	6	9.64
" <i>contorta</i> (Colo.).....	3 & 4	4.18	500	238	7	7	4	5.29
" <i>Banksiana</i> (Minn.).....	1 & 2	3.44	500	210	8	9	4	7.00
<i>Pseudotsuga taxifolia</i> (Wash.).....	2	8.77	500	177	5	16	7	16.6
" " (Colo.).....	4	9.58	500	310	7	19	11	14.1
<i>Abies lasiocarpa</i> (Mont.).....	2	7.01	500	4	1	—	—	4.0
" <i>grandis</i> (Mont.).....	3	15.20	500	0	—	—	—	—
<i>Tsuga mertensiana</i> (Mont.).....	1	2.08	500	0	—	—	—	—
<i>Thuja plicata</i> (Idaho).....	3	1.51	1,000	0	—	—	—	—
<i>Picea Engelmanni</i> (Colo.).....	All	3.18	500	312	9	6	1.5	4.06
<i>Sequoia sempervirens</i> (Calif.).....	4	2.41	500	55	3	8	5	6.00
" " (Calif.).....	2	3.30	500	63	7	11	2	7.33
" " (both)†.....					10	11	2	6.89

† Peters' abridged formula.

* Sown 27 days later than other species; samples of *P. Lambertiana* taken at a later date.

† The combined figures are used since the final weights of seedlings do now show much difference between the two lots.

demonstrate: it is not thought probable that the *relative* position of a given species would be appreciably affected by these qualities of the light, although it is possible that species accustomed to the strong ultra-violet of high mountains (e.g. Engelmann spruce) would suffer by comparison with those from low elevations.

Attempt was made to keep moisture and temperature conditions nearly optimum without any close regulation. As to the temperature effect of the light, this was found to be only 2° C. at the soil surface directly beneath the light, any further superheating being prevented by a fan which was directed across the table and was in constant use with the lamp.

A difficulty was experienced in interpreting the results of the earlier test, where it appeared that large seedlings persisted in weak light through use of food derived solely from their seeds, and hence appeared to possess "tolerance" which a longer period of starvation would have dissipated. To obtain a measure of the amount of growth in different light intensities, therefore, several seedlings of each species were taken up, dried, and weighed at the beginning of the period of artificial lighting. However, even this provision does not overcome the difficulties, as will be shown later, for seedlings growing under identical conditions show wide dispersion in their several weights, and any average based on a few samples contains a probable error of considerable magnitude (see table 2). Nevertheless, the average figures are much more dependable than those for individual trees, and it has been sought in the analysis of these data to compare average weights of trees surviving after the 9 months, as expressed by curves, with averages at the beginning, which are assumed to represent any point on the table.

At this initial stage, also, every seedling on the table which had had the benefit of sunlight was located as to its distance from the center. Intermediate records of losses and belated germination are not considered to have any great significance.

RESULTS

The significant facts were, therefore, obtained at the end of the test, by taking up every surviving seedling, and recording its position on the table and its dry weight. Root length and length of stem were likewise recorded, but these data add little information on the main point.

At the outset it seemed that it would be entirely feasible to plot the dry weights of the surviving seedlings as ordinates, and either the distances or the corresponding light intensities as abscissae, and thereby obtain for each species a curve of average seedling weights. The point, in distance or light intensity, at which this curve showed an average weight equal to the average weight of the seedlings at the beginning of the period of restricted lighting, would indicate the light intensity in which, and beyond which, no growth was made. This first point might well be denoted as the intensity of minimum effective light for the species.

In fact this simple method for determining the end-point can not be used with any high degree of precision, for two reasons:

1. As stated above, with normal variations in seed size there are wide variations in the weights of seedlings at any early stage, and the number of samples taken in this case was inadequate to fix the average weights for the species with errors of less than about 5 percent.

2. Even if the average weights were exactly known for the initial conditions of the exposure, the method would not work out correctly because of the fact, quite evident at the close of this test, that in many cases the survivors persisting in the weakest light had been originally the strongest and heaviest seedlings. For example, with Engelmann spruce the last six survivors (in point of distance) were all above the maximum weight of the original 9 samples.

This discussion is entered into for the purpose of recording this tendency for only the strongest seedlings to survive, and to point out the need, in an experiment of this kind, for equalizing the seedlings by grading the seed at least for size, and also for specific gravity if possible, thus insuring seedlings of reasonably uniform initial size and strength.

It would seem, then, that survival must be largely depended upon as the criterion of minimum light requirement. Here again, however, two obstacles are encountered: (1) the measure so obtained can not apply to the average seedling of a given lot, but only to those which were largest and strongest at the outset; (2) because of the limited period of light starvation the larger-seeded species possess an unnatural advantage, *i.e.*, they may be persisting without growth under conditions which would be fatal after a longer period. While both these qualities (large and strong seeds of exceptional character, and large seeds as a specific character) are doubtless important in the natural life struggle, it will probably be generally admitted that our interest is in the average or fairly typical representatives of the species.

It is, therefore, necessary to take into consideration several different expressions of tolerance or favorable reaction to weak light, in order to form a judgment as to what may be the minimum requirement of each species or a measure of absolute tolerance. The writers have no illusions as to their fallibility in weighing the data, nor as to the weakness of the data in several respects, and are partly encouraged to present these inconclusive results by the hope that others will note the number of preventable and of natural variables which enter into such a determination.

The various measures are shown in table 3 and their respective merits are discussed in the following paragraphs.

1. The percentage surviving, of the number of seedlings which obtained an early start in sunlight, can be only roughly suggestive of relative tolerance. As already pointed out, this might for a considerable time be influenced by the size of the seedlings, the extent to which they had become

TABLE 3. Comparative Results for Twelve Species Expressed by Various Measures of Survival and Growth

	Seedlings Living at End of Test		Average Light Intensity Enjoyed by Survivors (%)	Average Weight of Seedlings Growing in Light of 10% Intensity		Distances From Center:			Theoretical Minimum Light in Which Growth Could be Made (%)	Remarks on Determination of End-point
	Number	Ratio to Original Number† (%)		Actual* (mgs.)	Ratio to Original Average Weights	At Which Survival Was 10% of Original Number (inches)	Beyond 10% of Survivors Were Found (inches)	At Which Last Survivor Was Found (inches)		
Redwood (<i>S. sempervirens</i>).....	54	45.8	3.44	60.6	8.8 ±	37.9	33.0	37.2	0.62	Weight curve is fairly definite.
Engelmann spruce (<i>P. Engelmanni</i>).....	96	30.8	4.49	11.4	2.8	27.0	21.9	29.4	1.10	By weight curve, 100 or less; by survival curve 1.20%. Both very indefinite.
Douglas fir (Wash.) (<i>P. taxifolia</i>)	46	26.0	4.64	35.6	3.4	27.3	24.1	32.8	1.30	Last survivor given very little weight. Growth and survival curves agree quite closely.
Douglas fir (Colo.).....	58	18.7	4.52	25.0	1.8	23.8	22.4	24.2	1.50	Curves indefinite. Conclusion based on average position.
Yellow pine (Colo.) (<i>P. ponderosa</i>).....	70	17.1	5.37	94.0	3.6	23.2	19.4	22.9	1.80	Growth curve fairly definite, indicates 1.50%.*†
Yellow pine (Ariz.).....	77	24.7	5.26	132.0	3.4	24.4	21.9	29.7	1.80	Growth curve fairly definite, indicates 2.15%.*†
White pine (<i>P. Strobus</i>).....	11	22.4	4.73	18.4	2.3	—	22.0	22.1	2.00	Seedlings too few for definite determination. On average survival belongs lower in scale. Growth curve definite.
Jack pine (<i>P. Banksiana</i>)	40	19.0	5.24	19.8	2.8	22.6	21.5	22.7	2.38	Both curves very indefinite. Poor survival may not be due entirely to light conditions as indicated by good growth of survivors.
Lodgepole pine (<i>P. contorta</i>).....	15	6.3	6.08	24.2	4.6	—	19.5	19.6	2.40	Use survival curve; growth curve indefinite.
Norway pine (<i>P. resinosa</i>).....	61	19.6	6.34	21.2	2.2	19.9	17.5	20.1	2.55	Growth curve fairly definite.
Limber pine (<i>P. flexilis</i>).....	23	19.0	4.99	107.5	2.3	20.6	19.3	20.2	2.70	Growth curve fairly definite.
Piñon (<i>P. edulis</i>).....	37	21.5	6.00	133.0	1.3	21.3	18.6	20.6	6.30	Growth curve fairly definite. Survival influenced by large initial size of seedlings.

* No account taken here of germination after the exclusion of sunlight, since it is assumed most of such seedlings, or at least those in weak artificial light, were short-lived and did not enter into the final results at critical points.

† Discrepancy probably due to incorrect average weights. Note difference between seed and seedling weights in table 2. All measures not based on weight relations indicate the Arizona form to be more tolerant than the Colorado form. The figure 1.80% represents a compromise.

well-rooted on the food derived from the seed, and by such independent factors as damping-off fungi which are likely to flourish when physical conditions are not altogether favorable. This last factor, together with the slow germination of its seed, probably accounts for the poor showing of lodgepole pine, while limber pine and piñon would be especially helped by their large initial sizes.

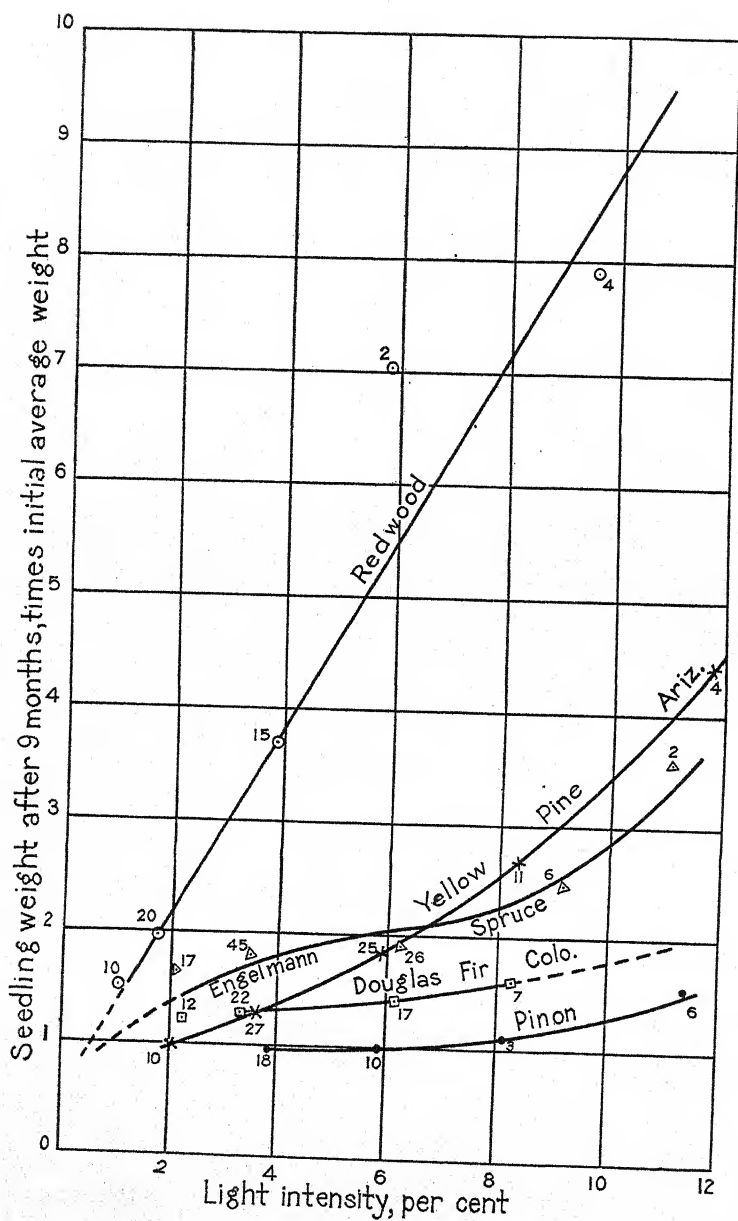
2. Practically the same objections may be raised against the average position and light intensity enjoyed by the surviving seedling, but because this measure takes into account *all* of the survivors, or an average response of the species, it must be given an unusual amount of consideration. The measure seems fairly satisfactory except in the case of limber pine where the original distribution was unfavorable; that is, there were very few seedlings near the center of the table, so that the final showing is strongly influenced by the large number at a distance.

It is well to point out in this connection that there was a tendency to sow somewhat more thickly near the center than at the edge of the table, having in mind that seedlings at the center would interfere with one another less because of the more vertical angle of the light in this region.

3. The distance of each seedling from the center of the table has been translated into an expression of light received by it, and its weight has then been plotted, using the light intensities as the abscissae. The curve of weights or growth has usually been drawn after determining four or five average or group points, obtained by dividing the light intensities into as many groups, that is, 0-2.50 percent, 2.51-5.00 percent, etc.

Nearly all of these curves are sufficiently definite to permit a close determination of the average size attained by seedlings in light of 10-percent intensity, that is, near the center of the table. Relating this weight to the original average weight for the species gives an expression useful for comparative purposes, so far as the original average can be depended upon. And it must be realized, also, that this expression of the ability to utilize light is very greatly influenced by the habit of the species. Thus, there can be no comparison, as a measure of tolerance alone, between the two coastal species (redwood and Douglas fir) accustomed to very favorable growing conditions, and most of the Rocky Mountain species accustomed to having to protect themselves against unfavorable growing conditions. Thus, the habit of piñon is very marked. It produces a deep root even before the cotyledons emerge from the seed-coat, and under the most favorable conditions makes no visible growth for some time after producing a few primary needles. Of the Rocky Mountain species lodgepole stands at the other extreme, being known as a "quick grower" when conditions are favorable.

This measure, then, can be only slightly suggestive. It is desirable to point out, however, that in nearly all cases the seedling size attained in 10-percent light for nine months under these conditions is just about equal



TEXT FIG. 1. Weights of seedlings produced in various intensities of light, and varying responses of the species to the light scale as influenced by inherent habit. Numbers near points indicate numbers of seedlings entering into the averages.

to that usually attained at the end of a year in full sunlight. Moreover, the seedlings growing in 10 percent light were not noticeably abnormal in appearance or proportions, though probably a little more leafy and less firm than seedlings grown under natural conditions.

As measures of the end-points of tolerance, these same growth curves may be used in a few instances, although qualifications are necessary. For the most part it must be assumed that the few seedlings growing in the weakest light are not truly representative as to size, and the growth curve must be drawn below the last point if possible. The critical portion of the curve is, therefore, indefinite, except as the direction of the entire curve is clearly fixed by other points. A few of the curves and their difficulties are illustrated in text figure 1. The curve for redwood, which can be drawn as a straight-line correlation between light intensity and weight of seedlings, best illustrates the principles which we have attempted to follow, while the curve for Engelmann spruce shows the impossibility of any accurate determination of end-point when the relationship is irregular.

It is impossible to offer adequate explanation of the tendency most noticeable with Engelmann spruce, but also evident with both lots of Douglas fir, and with Jack and lodgepole pines, to show less relative weight accretion in an intermediate zone than at either extreme. However, in view of the fact that these are all short-stemmed species, producing little growth in this period beyond the cotyledons, it is suggested that their irregular performance might be due to light interference by the larger trees in the zone where the survival was generally good. It is also probable that the middle zone was somewhat cooler than either the center or edge of the table, the latter being more directly warmed by wall radiators, and the former by the light. This, however, should have affected all species alike.

4. The survival curves have been plotted in much the same way as the growth curves, but after grouping the original and surviving trees in 2-inch zones. From such curves, irregular as some of them may be at the limits of survival, we may read off the approximate distance (and light intensity) at which 10 percent of the trees might be expected to live, regardless of the amount of growth made. This appears to be a fair basis for comparing the species, though not wholly satisfactory because of irregularities in original distribution and the small number of trees involved at the critical points.

5. The point at which the last survivor was found obviously must be influenced by some exceptional conditions if this survivor stands very much alone, as is frequently the case. This is somewhat less true if we draw the line inside of 10 percent of the survivors, where we have the evidence of from two to eight seedlings.

CONCLUSIONS

1. Considering all of the measures which can be used in the interpretation of these data, it is evident that there must be wide variations in the

ability of the different species to use weak light of the quality furnished in this experiment. With a minimum requirement of less than three-fourths of one percent, and increasing its original size almost ten times in light of 10 percent intensity, redwood stands out as by far the most efficient mechanism for photosynthesis. Such a result might readily be inferred from its enormous capacity to produce wood under its natural growing conditions.

2. Engelmann spruce and the two forms of Douglas fir fall not far behind redwood though perhaps requiring twice as much light for appreciable growth. Neither in this nor any other tests has it been possible to distinguish clearly an advantage of one of these species over the other.

The pines, in general, fall in a group requiring three to four times as much light as redwood, while piñon stands quite apart from the others, requiring an intensity of 5 percent or more. Such a relative light demand might be expected from the fact that the moisture conditions under which piñon normally grows never permit anything but the most open stands and open-crowned trees. It has never had to be efficient in the use of sunlight, where moisture is clearly limiting.

3. There is sufficient difference in growth responses under the conditions of this experiment, if not in absolute light requirements, to show plainly the extent to which geographic forms or varieties of the same species develop individual physiological characters. The growth curves for Colorado and Arizona yellow pine are quite similar, as might be expected, while the Coast form of Douglas fir, though more active and responsive on the whole than the Colorado form, is relatively much less responsive to very weak light. There may be a temperature response involved in this difference.

4. The extent to which a few individuals of several of the species, presumably those of greatest initial size and vigor, stand out above the mass in photosynthetic efficiency or at least in survival in weak light, suggests the possibility of utilizing conditions such as obtained in this experiment for the artificial selection of a strain possessing high efficiency. This would not be essentially different from the natural selection which is constantly occurring in forests of high density, except as it be admitted that in nature the light intensity or quantity is rarely so low that it is likely to be the limiting factor. It is desired to point out, as in the earlier paper already referred to, that actual failure to synthesize and produce some growth is not so likely to be fatal to seedlings as the fact that slow growth, and especially insufficient food for the roots, leaves the seedling unprepared to cope with the surface drying of the soil, such as is more or less normal toward the close of any growing season.

ROCKY MOUNTAIN FOREST EXPERIMENT STATION,
COLORADO SPRINGS,
COLORADO

GENERA OF NORTH AMERICAN FABACEAE
III. TRIBE PSORALEAE

P. A. RYDBERG

(Received for publication August 12, 1927)

The tribe was based on Bentham and Hooker's subtribe *Psoralieae* of the tribe *Galegeae*. (See Benth. & Hook. Gen. Pl. 1: 443). It was characterized as follows:

Glandular-punctate herbs or shrubs. Racemes or spikes terminal or axillary. Anthers muticous. Ovules 1-2 or rarely 3-4. Legume small, often 1-seeded indehiscent.

Taubert in Engler and Prantl's *Natürlichen Pflanzenfamilien* followed their classification, but changed the name of the subtribe to *Psoraliinae*. He characterizes it as follows:

Connection without appendages. Ovules 1-2, very seldom 3-4; pod small, mostly with only 1 seed, indehiscent; glandular-dotted herbs or shrubs.

Both Bentham and Hooker, and Taubert, admit that these characters do not absolutely distinguish the genera of this subtribe from all other genera of *Fabaceae*, for 1-seeded indehiscent pods are found elsewhere in the family and glandular-dotted plants are also found, as *Glycyrrhiza*. They have, however, overlooked the fact that several of the species by them included in *Psoralea* have dehiscent pods. This is the case with all those segregated by me as the genus *Pedimelum*. I also suspect that in the genus *Hoita*, judging from the texture and structure, the pod also is tardily dehiscent. If so it is valvate, not circumscissile as in *Pedimelum*.

Though there remains not a single character that absolutely distinguishes the genera of this tribe from all others in the family, the group is rather natural and in my opinion it should be regarded as a tribe rather than a subtribe of *Galegeae*. It shows, for instance, just as much affinity to *Hedysareae* or *Trifolieae*, in which tribes 1-seeded indehiscent pods are found. In *Cullen* (a segregate from *Psoralea*), the leaflets are toothed and the pod much like that found in the latter tribe, while in *Asphaltium* the pod is practically a loment of 2 internodes, the lower closely investing the seed and the upper (the beak) empty and breaking off.

The tribe shows many variations, and the genera show more abnormalities than in any other tribe as far as I know. The tribe includes herbs, shrubs, and trees, usually with gland-dotted foliage. In some cases the gland-dots are confined to the calyx or fruit alone. In one case, *Or-*

bexilum stipulaceum (T. & G.) Rydb., the calyx has no glands and the fruit is unknown, and the plant may not belong to the tribe. Perhaps it represents a species of *Meibomia*. Both odd-pinnately and palmately compound leaves are found; in some cases they are reduced to a single leaflet.

The flowers are perfect, in spikes, racemes, or panicles. The calyx is usually campanulate, 5-toothed or 5-lobed, the lowest tooth or lobe usually somewhat longer. In *Apoplanesia* it is almost rotate, cleft to near the base and enlarging in fruit.

The corolla is usually more or less papilionaceous of 5 petals, of which the two lower are more or less united. In *Parryella* it is wanting, in *Amorpha* reduced to the banner, and in *Eysenhardtia*, *Apoplanesia*, and *Psorobatus* consisting of free slightly differentiated petals. So are the petals also in *Kuhnistera*, *Petalostemon*, and *Thornbera*, but their claws (except that of the banner) are adnate to the staminal sheath. In *Parosela* they are also more or less adnate to the tube, but the corolla is more papilionaceous. In *Psorodendron* and *Psorothamnus* they are also wholly free but decidedly differentiated.

The stamens are usually 9 or 10, in a few species of *Parosela* 7 or 8, in *Petalostemon* and *Kuhnistera* only 5. In *Amorpha*, *Apoplanesia*, and *Parryella* they are distinct to near the base. In the rest, all of the filaments are united into a long sheath, split on the upper side, if the number is less than 10; the tenth filament, if present, is usually free.

The ovary is usually 1- or 2-ovuled, but *Psorodendron spinosum* has sometimes as many as 6 ovules; the style is slightly bent and the stigma minute.

The fruit is mostly 1-seeded, sometimes 2-seeded and indehiscent. The greatest variation in the fruit is found in *Psoralea*, as the genus is generally understood. Bentham and Hooker characterized the fruit of that genus as having an indehiscent 1-seeded pod with the seed adherent to the pericarp. Taubert repeats these characters. None of our American species have such a fruit. In those segregated by me as the genus *Pedionelum* the fruit is dehiscent, either circumscissile or bursting irregularly near the middle. The style is long and remains as a stout beak. When the pod is ripe and this beak is touched, it snaps the pod, carrying with it the upper part of the ovary, and releases the seed. In the segregate *Hoita* the pericarp is loose and thin. It may be dehiscent tardily. In the rest of the American segregates the pod is truly indehiscent with a hard, woody or bony pericarp but in none is it adherent to the seed. I doubt if the seed is adherent to the pericarp in any of the typical species of South Africa. It is found in two groups native to the Mediterranean region and India. These are represented, one by *Psoralea bituminosa* L. and *P. plumosa* Reichenb., the other by *P. americana* L. and *P. corylifolia* L. As these two groups were better known to European botanists, they probably furnished the characters on which these botanists based their concept of

the genus. The type of the genus, however, is a South African species, *P. pinnata* L., and the genus should be limited to that species and its relatives, all South African.

Medicus, who showed much acuteness in segregating genera where his predecessors had retained rather unrelated groups in the same genus, saw that *Psoralea* was a conglomerate, and divided it in his Vorlesungen into 4 genera, of which *Asphalthium* and *Cullen* represented the two groups mentioned above. Unfortunately he applied the name *Psoralea* to the group represented by the South American *P. glandulosa* L. and proposed the name *Ruteria* for the South African group of true *Psoralea*. Medicus, however, was not the only botanist who saw the complexity of the genus. Moench called *Cullen* *Dorychnium* and Presl used the name *Rhynchodium* for *Asphalthium*. Rafinesque segregated one of the groups endemic to North America under the name *Orbexilum*.

The genus *Parosela* Cav. (*Dalea* Vent. in part, not Mill.), as usually understood, is also a complex genus. In the subgenus *Xylodalea* of Watson the petals are not "inserted on the staminal tube" but in the bottom of the calyx on a small disk as usually in Fabaceae, and it therefore should not be included in the genus *Parosela*. The subgenus is not homogenous, however, but represents 3 distinct groups, to which I have given generic rank. Some of the species of the section *Eudalea* of southwestern United States and Mexico have been removed from the genus and collected in a new genus *Thornbera* as the flower is essentially that of *Petalostemon* except that the stamens are 10 instead of 5.

The following key may serve in distinguishing the genera:

Petals distinct from the staminal tube, inserted on the hypanthium.

Ovary 1-ovuled and pod 1-seeded.

Calyx-lobes not much enlarging in fruit, erect; corolla papilionaceous.

Pod indehiscent.

Plants shrubby, with spinescent-tipped leaflets; leaves odd-pinnate with a sessile terminal leaflet or 3-foliolate.

Plants herbaceous or shrubby, with innocuous leaflets.

Pericarp adherent to the seed; leaves pinnately 1-3-foliolate; Old World genera.

Pod conspicuously glandular-warty, with a short persistent beak; leaflets coarsely dentate.

Pod not conspicuously warty, with a long flat beak, which ultimately breaks off; leaflets entire.

Pericarp not adherent to the seed; American genera.

Leaves pinnately 3-foliolate.

Pod coriaceous or bony, cross-wrinkled, neither hairy nor included in the calyx.

Pod membranous or leathery, not cross-wrinkled, hairy, included in the calyx.

1. PSORALEA.

2. CULLEN.

3. ASPHALTHIUM.

4. ORBEXILUM.

5. HOITA.

Leaves digitately compound.

Pod crescent-shaped, flat, cross-ribbed; claw of banner twice bent.

Pod orbicular to oblong-ovate, slightly compressed, glandular-dotted but not cross-ribbed; claw of banner straight to the base of the blade.

Pod circumscissile or bursting irregularly; beak long; leaves digitately compound, or the median leaflet petioled, and if 3-foliolate apparently pinnate.

Calyx-lobes much enlarging and spreading in fruit, reticulate-veined; corolla scarcely papilionaceous, all petals distinct; shrubs, with odd-pinnate leaves and many leaflets.

Ovary 2-6-ovuled.

Filaments distinct, or united only at the base, corolla wanting or reduced to the banner only.

Banner wanting.

Banner present.

Filaments united more than half their length into an elongate tube split on the upper side; corolla usually present.

Pod flat, exserted; stamens diadelphous.

Pod turgid; stamens monadelphous.

Corolla scarcely papilionaceous; petals all free and nearly clawless; stipules spinescent.

Corolla papilionaceous; petals clawed and those of the keel usually adnate or coherent.

Pod exserted; flowers pedicelled.

Pod not exserted; flowers sessile.

Petals, except the banner, adnate to or inserted on the staminal tube.

Wings and keel-petals distinctly clawed, inserted some distance below the mouth of the staminal tube, the keel-petals inserted higher up than the wings, the former usually united along the lower edge of the blades.

Wings and keel-petals alike, all distinct and inserted at the mouth of the staminal tube; spikes dense, with persistent bracts.

Stamens 10 or rarely 9.

Stamens 5.

Banner much broader than the other petals; calyx-lobes not much if at all exceeding the tube; spikes not involucrate.

Banner similar to the other petals but long-clawed; calyx-lobes about 3 times as long as the tube, plumose; spikes involucrate, *i.e.*, subtended by many conspicuous empty bracts.

6. RHYTIDOMENE.

7. PSORALIDIUM.

8. PEDIOMELUM.

9. APOPLANESIA.

10. PARRYELLA.

11. AMORPHA.

12. EYSENHARDTIA.

13. PSOROBATUS.

14. PSORODENDRON.

15. PSOROTHAMNUS.

16. PAROSELA.

17. THORNBURA.

18. PETALOSTEMON.

19. KUHNISTERA.

1. *Psoralea* [Royer] L. Spl. Pl. 762. 1753

Shrubs, with densely leafy stems, conspicuously glandular-dotted. The leaves are odd-pinnate with a sessile terminal leaflet or sometimes 3-foliolate, the leaflets are usually spinulose-tipped. The flowers are axillary or in terminal sessile spikes. The calyx is strongly glandular-punctate and strongly ribbed, 5-lobed. The corolla is usually longer than the calyx; the petals are strongly veined, the banner free, broadly obovate, the margins

spreading and usually with two rounded lobes above the short claw; the other petals have longer claws, and obliquely obovate or lunate blades with a distinct auricle on the upper side. The filaments of the nine stamens are united, the tenth if present almost free or more or less partly united with the rest. The pod is oval or ovate, indehiscent, short-beaked, the pericarp free from the kidney-shaped seed.

ILLUSTRATION: Plate XII A. *Psoralea pinnata* L., $\times 2/3$; 1. leaf; 2. calyx, spread open, $\times 1$; 3. calyx, side-view, $\times 2$; 4. banner; 5. wing; 6. keel-petal, $\times 1$; 7. pod; 8. pod, in cross-section; 9. seed, $\times 2$; 10. stamens; 11. pistil, $\times 2$.

The genus was instituted on 8 species, and adopted from Royen. Of these only two, *P. pinnata* and *P. aculeata*, were known to that author. Linnaeus himself had treated the first of these under the name *Psoralea* in both Hortus Upsaliensis and Hortus Cliffortianus, and it must therefore be regarded as the type. *P. aculeata*, as well as Linnaeus's third species, *P. tenuifolia*, belongs in my opinion to the same genus.

SYNONYM:

Rutaria Medic. Vorles. Churpf. Phys.-Oek. Ges. 2: 381. 1887. Type: *Psoralea pinnata* L., the only species.

The genus consists of perhaps 40 species, all natives of South Africa, one of these, *P. fruticans* (L.) Rydb. (*P. bracteata* L.), has been collected adventive on Mt. Tamalpais, California. The typical species of the genus are shrubby, with solitary or fascicled axillary flowers, conspicuously striate-veined petals, and small indehiscent pods, neither bony nor reticulate. Whether all the African species should be included in the genus is very doubtful and further segregation may be necessary. A few species may belong to the genera, now wholly regarded as American. A few of them have a habit somewhat resembling *Psoralidium*, but the fruit is unknown to me.

2. Cullen Medic. Vorles. Churph. Phys.-Oek. Ges. 2: 380. 1787

Annual or perennial herbs. The leaves are pinnately 1-3-foliolate, conspicuously glandular-dotted, with coarsely dentate leaflets. The flowers are in peduncled axillary spikes. The calyx is strongly glandular-punctate; the tube campanulate, not gibbous, 5-lobed, the lowest lobe usually the longest and the uppermost two more or less united. The corolla is scarcely longer than the lowest calyx-lobe, the banner is obovate with a very short claw, the margins merely spreading; the wings have rather narrow oblong blades with a reflexed large basal auricle; the keel-petals have broadly obliquely lunate blades united at the rounded apex and slightly united with the adjacent wing at the base. Nine of the filaments are united into a 9-toothed sheath, the tenth free or nearly so. The style is slightly curved, the stigma small, capitate. The pod is glandular-warty, with an erect beak, the pericarp thin, adherent to the obliquely reniform seed.

ILLUSTRATION: Plate XII B. *Cullen americanum* (L.) Rydb. $\times 2/3$; 1. calyx; 2. banner; 3. wing; 4. keel-petal; 5. stamens; 6. pistil, $\times 3$; 7. pod of *C. corylifolium* (L.) Rydb.; 8. pod in cross-section; 9. seed, $\times 2$.

The type of the genus is *Psoralea corylifolia* L. the only original species.

SYNONYMS:

Dorychnium (Royen) Moench. Meth. 109. 1794. Moench adopted the pre-Linnaean name given by Royen and cites *Psoralea corylifolia* L. and the generic name *Cullen* Med.

Lotodes (Siegesbeck) Kuntze, Rev. Gen. 193. 1891. Kuntze adopted the pre-Linnaean name of Siegesbeck changing the spelling from *Lotoides*. He also claimed that Siegesbeck had in mind *Psoralea corylifolia* L.

The genus consists of at least 2 species, *Cullen corylifolium* (L.) Medic. with 1-foliolate leaves, a native of the East Indies, and *C. americanum* (L.) Rydb. with 3-foliolate leaves, of Madeira, Spain, and northeast Africa; and adventive from Florida to Mississippi and in Cuba. Three or four Australian species may also belong here. The genus is distinguished from *Psoralea* by the herbaceous habit and the structure of the pod and from all the other segregates here treated by the dentate leaflets.

3. *Asphalthium* Medic. Vorles. Churpf. Phys.-Oek. Ges. 2: 380. 1787

Glandular-dotted herbaceous perennials. The leaves are pinnately trifoliolate. The flowers are borne in head-like spikes on elongate axillary peduncles. The calyx is inconspicuously or obscurely punctate, 5-cleft; the tube is campanulate, the lobes unequal and the lowest much longer than the rest. The banner is narrow, oblanceolate and sagittate at the base with a broad claw; the wings have a semi-sagittate blade, longer than the claw and slightly adnate to the keel-petals; the keel-petals have an oblong-oblanceolate blade shorter than the claw. The stamens are 10, monadelphous at the base, but the tenth stamen free for most of its length. The pod is indehiscent, with the pericarp adherent to the seed and with a sword-shaped beak, which ultimately breaks off.

ILLUSTRATION: Plate XII C. *Asphalthium bituminosum* (L.) Kuntze, $\times 1/2$; 1. calyx, $\times 2$; 2. banner; 3. wing; 4. keel-petal, $\times 1$; 5. stamens; 6. pistil; 7. pod; 8. pod in cross-section; 9. seed, $\times 2$.

The genus was based on a single species, *Asphalthium frutescens* Medic., which is the same as *Psoralea bituminosa* L.

SYNONYM:

Rhynchodium Presl. Bot. Bemerk. 54. 1846, based also on *P. bituminosa*.

The genus consists of the type and 3 or 4 closely related species of the Mediterranean region. The general habit of the plants is that of a clover, and it is known in English as "asphalt clover." The pod, as stated before, suggests a relationship with the tribe Hedysarieae, in which the loment consists of two joints, of which the upper is empty.

4. *Orbexilum* Raf. Atl. Jour. 145. 1832

Perennial herbs, with rootstocks. The leaves are usually glandular-dotted, pinnately 1-3-foliolate, rarely 3-foliolate. The flowers are borne in axillary long-peduncled spikes or racemes. The calyx is campanulate, usually glandular-dotted, 5-lobed. The corolla is blue or purple, not

strongly veined; the banner is short-clawed, broadly obovate, with recurved sides; the wings are slightly longer than the banner, with an obliquely lunate blade and a basal auricle; the keel-petals are similar, but shorter, united at the tip and the blades adnate to those of the wings at the base. The stamens are diadelphous, *i.e.*, the tenth stamen wholly or partly free, or rarely monadelphous. The style is slightly curved, the stigma capitate. The pod varies from suborbicular to obliquely ovate, is included in the calyx, somewhat compressed, indehiscent, with an incurved beak; the pericarp is thick, wrinkled or ribbed, often tuberculate, thick, not coherent to the suborbicular or reniform seed.

ILLUSTRATION: Plate XII D. *Orbexilum Onobrychis* (Nutt.) Rydb. $\times 2/3$; 1. calyx, $\times 4$; 2. banner; 3. wing; 4. keel-petal, $\times 2$; 5. stamens; 6. pistil, $\times 4$; 7. pod; 8. pod in cross-section; 9. seed; 10. pod of *O. virgatum* (Nutt.) Rydb.; 11. seed, $\times 2$.

The genus was based on *Psoralea latifolia* Torrey, which is the same as *P. Onobrychis* Nutt. It consists of 8 species, one of these a native of Mexico, the rest of eastern United States. They may be divided into two groups of 4 each, *Euorbexilum*, with obliquely ovate pod, broad leaflets and rootstocks, and *Poikadenia*, with suborbicular pods, narrow leaflets and fusiform roots. The latter constituted the *Psoralea* § *Poikadenia* Ell. (Sketch Bot. S. C. & Ga. 2: 197. 1822). The genus differs from *Psoralea* principally in the herbaceous habit, the pinnately 3-foliolate leaves, the coriaceous or bony, and reticulate or even tubercled fruit.

5. *Hoita* Rydb. N. A. Fl. 24; 7. 1919

Perennial herbs with rootstocks, rarely shrubby below. The leaves are pinnately 3-foliolate, with entire, short-petioluled, conspicuously glandular-punctate leaflets. The flowers are borne in long-peduncled, axillary spikes or racemes. The calyx is campanulate, not gibbous, glandular-dotted, 5-lobed, the lowest lobe the longest. Corolla purple or ochroleucous, the keel with a dark-purple tip; the banner is broadly obovate or suborbicular, with a short curved claw; the wings have obliquely oblong blades auricled on the upper side at the base; the keel-petals are similar but the blade more lunate. The stamens are diadelphous or the tenth one united with the sheath at the base. The style is abruptly bent above the middle, the stigma capitate. The pod is obliquely ovate or oval, with a slender beak, pubescent; the pericarp is thin, free from the oval-reniform smooth seed, somewhat reticulate but not ribbed.

ILLUSTRATION: Plate XIII E. *Hoita macrostachya* (DC.) Rydb. $\times 2/3$; 1. calyx, $\times 2$; 2. banner; 3. wing; 4. keel-petal, $\times 1$; 5. stamens; 6. pistil; 7. pod; 8. pod, in cross-section; 9. seed, $\times 2$; 10. banner in side view, $\times 1$.

As the type *Psoralea macrostachya* DC. was designated. The genus contained originally 11 species, all from the Pacific slope of America, 10 natives of North America and the eleventh of Peru to Chili and introduced in California. To this genus apparently belong also all the South American species described in *Psoralea* and one or two Australian. The genus resembles closely the preceding in habit but differs in the structure of the

pod, which is thin-walled and may be at last dehiscent. Among the American segregates, this genus approaches in fruit mostly the Old World *Psoralea*.

6. *Rhytidomene* Rydb. N. Am. Fl. 24; 12. 1919

Perennials, with a creeping rootstock. The leaves are digitately 5-7-foliolate, with glandular-dotted linear-filiform leaflets. The flowers are borne in long-peduncled axillary lax racemes. The calyx is campanulate, with short tube, 5-lobed, the lowest lobe the shortest. The corolla is blue, veiny; the banner has a conduplicate suborbicular blade and a doubly bent short claw; the blade of the wings is obliquely obovate, with a small auricle at the base; the keel-petals are shorter, with a lunate blade and scarcely any auricle; they are united at the rounded apex and slightly coherent with the blades of the wings. The filaments are diadelphous, *i.e.*, the upper one wholly free. The pod is crescent-shaped, somewhat twisted, exserted from the calyx. The pericarp is coriaceous and obliquely cross-wrinkled.

ILLUSTRATION: Plate XIII F. *Rhytidomene Lupinellus* (Michx.) Rydb. $\times 2/3$; 1. calyx, $\times 4$; 2. and 3. banner; 4. wing; 5. keel-petal, $\times 2$; 6. stamens; 7. pistil, $\times 4$; 8. pod; 9. pod in cross-section; 10. seed, $\times 2$.

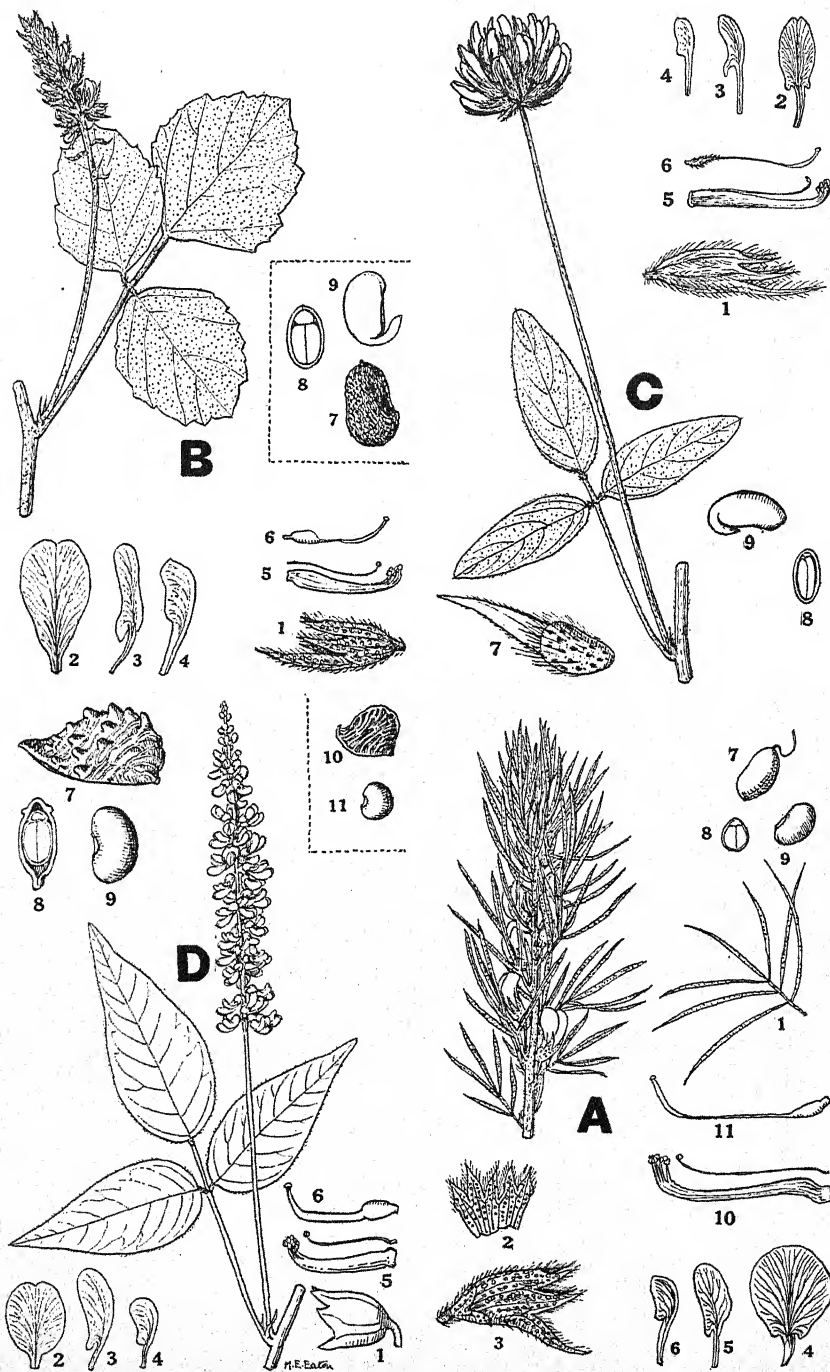
The genus was based on a single species, *Psoralea Lupinellus* Michx., of the southeastern United States. It is monotypic unless one or two Australian species may be included in it; of these no fruit has been seen. The fruit of *Rhytidomene* resembles most that of *Orbexilum*, but the genus differs from the latter in the palmately compound leaves and the doubly bent claw of the banner.

7. *Psoralidium* Rydb. N. Am. Fl. 24; 12. 1919

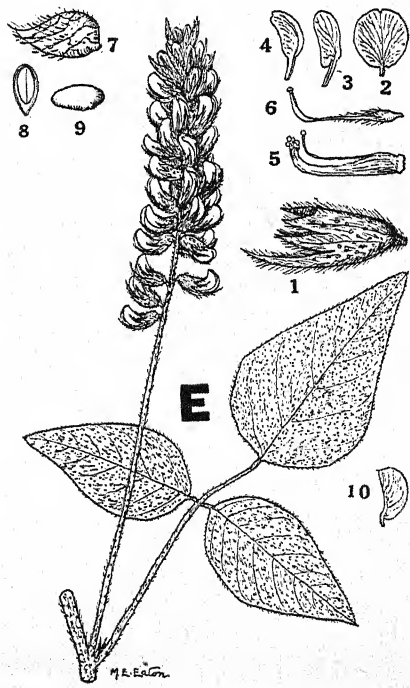
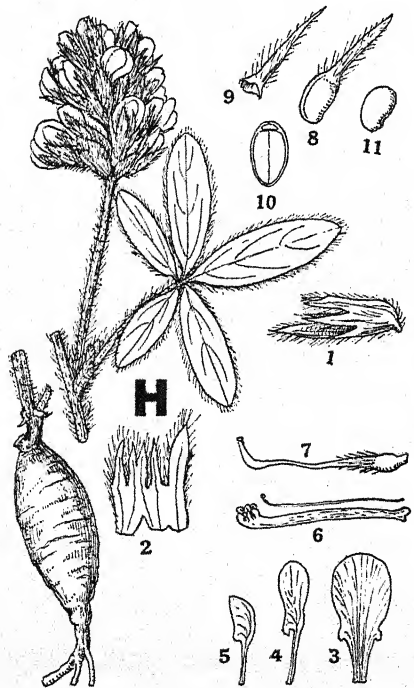
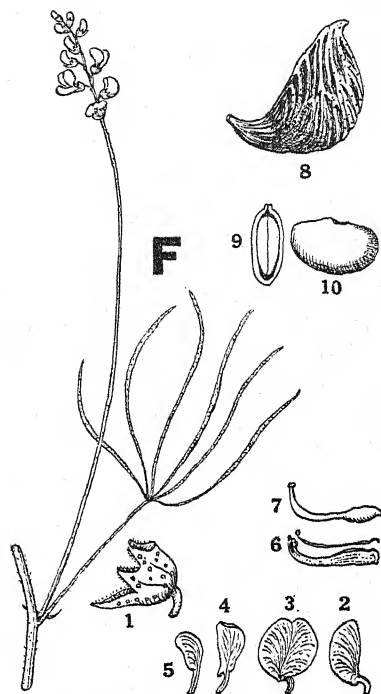
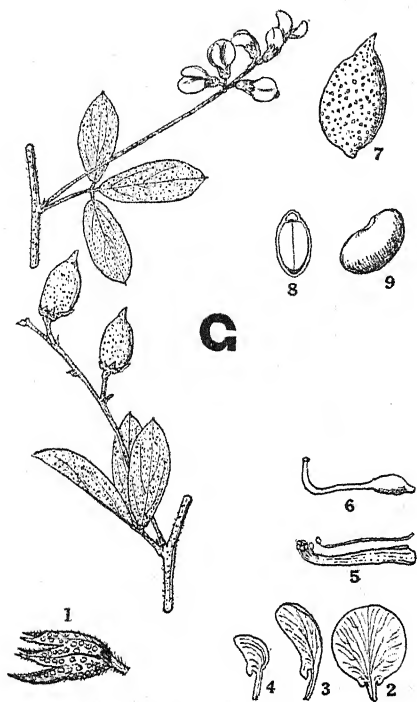
Perennial herbs, with rootstocks. The leaves are usually strongly glandular-dotted, digitately 3-5-foliolate. The flowers are borne in interrupted spikes or racemes, bearing 1-4 flowers at each node. The calyx is campanulate with a short tube, 5-lobed, the lowest lobe usually slightly longer than the rest. The corolla is blue, purple, or rarely white, with purple-tipped keel; the banner is rounded-obovate or suborbicular, with a short claw and usually with 2 small basal auricles. The wings and keel-petals have blades slightly united with their neighbors at the base, and in the case of the latter, at the apex; the blades are obliquely oblanceolate, more or less falcate or lunate, with a basal auricle. The keel-petals are shorter than the wings. The filaments are diadelphous, the tenth filament wholly free. The fruit is indehiscent, 1-seeded, short-beaked, somewhat compressed, ovate or orbicular in outline, usually copiously glandular-dotted, with a coriaceous pericarp free from the kidney-shaped seed.

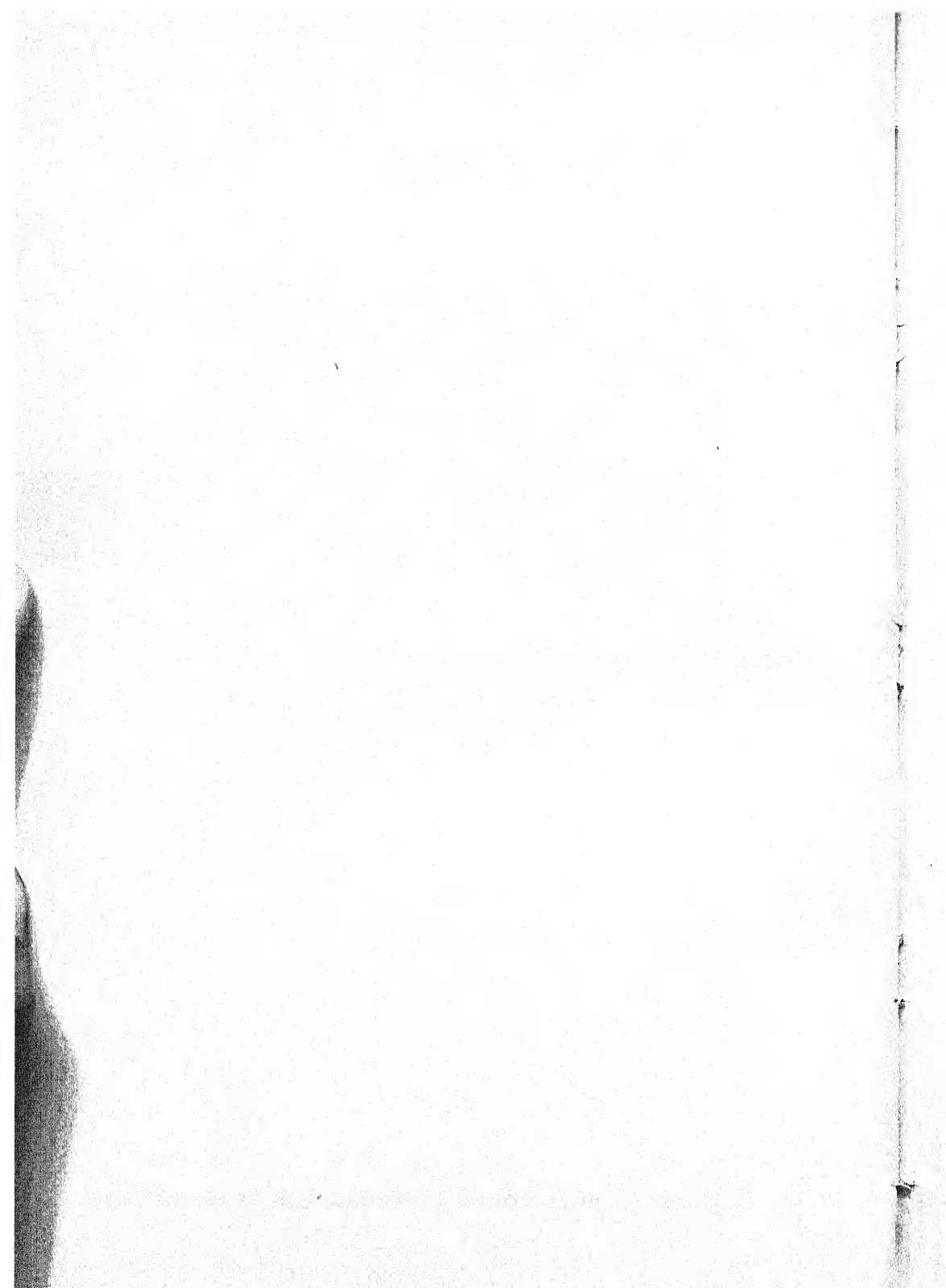
ILLUSTRATION: Plate XIII G. *Psoralidium tenuiflorum* (Pursh) Rydb. in flower and fruit, $\times 1$; 1. calyx, $\times 4$; 2. banner; 3. wing; 4. keel-petal, $\times 2$; 5. stamens; 6. pistil, $\times 4$; 7. pod; 8. pod in cross-section; 9. seed, $\times 2$.

Psoralea tenuiflora Pursh was assigned as the type. The genus as originally instituted consisted of 14 species natives of the prairies and plains of western North America, only one species extending into Northern



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Mexico. Whether any of the African species of *Psoralea* should be included in this genus is doubtful. None are represented in the herbarium of the New York Botanical Garden. As far as the fruit is concerned, the genus is closer to the original *Psoralea* than any of other segregates, and hence the selection of the name *Psoralidium*; but the general habit is quite different, the leaves digitate, never spinulose tipped. The inflorescence is always axillary, interruptedly spicate or racemose, with the flowers fascicled at the nodes and the corolla never strongly striately veined, as in the typical *Psoraleae*.

8. *Pedionelum* Rydb. N. Am. Fl. 24; 17. 1919

Perennials, with deep-seated, rounded or fusiform, tuberous, farinaceous, edible roots. The leaves are usually digitately 3-7-foliolate or in some species the middle leaflet is petioluled. The flowers are borne in axillary peduncled spikes or racemes. The calyx is gibbous on the upper side at the base, 5-lobed, with the lowest lobe the longest. The corolla is blue or purple. The banner is broadly obovate or oblanceolate, tapering into the claw, which is decidedly channeled; the wings are nearly as long, the blade oblong-oblanceolate, with a recurved basal auricle; the keel-petals are shorter, the blade lunate, slightly adnate to the adjacent wing, with a slender claw. The filaments are diadelphous, *i.e.*, with the tenth filament wholly free. The style is abruptly bent near the apex, the stigma capitate. The pod has an oval compressed body and a sword-shaped beak, is circumscissile or irregularly bursting around the middle, the pericarp being thin and loose, the upper part falling off with the beak.

ILLUSTRATION: Plate XIII H. *Pedionelum esculentum* (Pursh) Rydb. $\times 2/3$; 1. calyx; 2. the same laid open; 3. banner; 4. wing; 5. keel-petal, $\times 1$; 6. stamens; 7. pistil, $\times 2$; 8. pod; 9. beak of the same broken off at maturity, $\times 1$; 10. pod, in cross-section, $\times 2$; 11. seed, $\times 1$.

Psoralea esculenta Pursh was regarded as the type. The genus is wholly North American, and consists of 22 species. It was divided into two sections: *Eupedionelum* with 16 species having truly digitate leaves with sessile leaflets, and *Geomelum* of 6 species with the median leaflet petioled. The former are natives of western United States and southwestern Canada, only one species extending into Mexico; the latter are confined to Mexico, only one species extending into Texas and Louisiana. The genus differs from the other segregates of *Psoralea* in the dehiscent pod. This resembles somewhat that of *Asphalthium*; in the latter genus only the beak breaks off leaving the seed enclosed in the adherent and indehiscent pericarp. The tuberous farinaceous roots are also characteristic of *Pedionelum* and found elsewhere in the tribe only in the section *Poikadenia* of *Orbexilum*.

STUDIES IN THE CHYTRIDIALES II. CONTRIBUTION TO
THE LIFE HISTORY AND OCCURRENCE OF *DIPLO-
PHLYCTIS INTESTINA* (SCHENK) SCHROETER
IN CELLS OF AMERICAN CHARACEAE

J. S. KARLING

(Received for publication August 22, 1927)

The life history of *Diplophlyctis intestina* is well known from the excellent studies by Schenk and Zopf, and is similar in most respects to that of *Entophlyctis heliomorpha*, another member of the Rhizidiaceae with which it is frequently associated in dead cells of the Characeae. Its essential structural differences lie in the presence of an enlargement at the base of the sporangium, the apophysis, from which the highly developed and richly branched rhizoidal or hyphal system originates; and in the formation of a spiny- or rough-walled resting spore. As has been described by Schenk and Zopf the zoospore produces a germ tube which penetrates the wall of the host cell and forms a globular sporangium at its tip. The sporangia bear from one to more than a hundred zoospores which escape to the outside of the host through a sporangial neck. These zoospores germinate and continue to reinfect the host cell, until it is filled with sporangia. Several long internodal cells of *Nitella* and *Chara* have been found with as many as five thousand mature sporangia. Resting spores are formed from zoospores in the same host cell in the same fashion as are the sporangia.

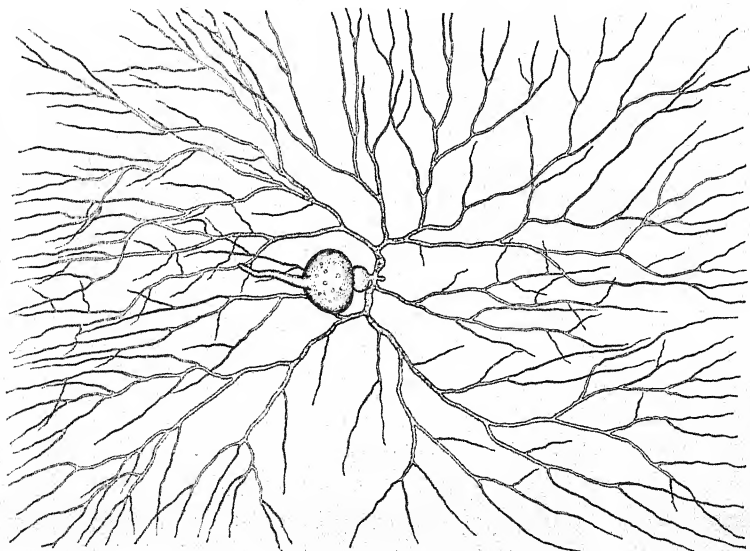
The first description of this chytrid was given by Schenk in 1858 in which he placed it in the genus *Rhizidium* under the name of *R. intestinum*. Schenk reported it as occurring in great abundance in internodal cells of *Nitella flexilis* and figured the germination of the zoospores and the development of the sporangium, but he did not observe the resting spores. In 1884 Zopf gave a complete account of its occurrence in cells of *N. flexilis* and *N. mucronata* and figured several stages of its life history, including the formation of the resting spores. Dangeard reported its occurrence in cells of *N. tenuissima* and *Chara polycantha* in 1886 and figured a few mature sporangia and escaping zoospores. In 1890 he reported it again and figured the development of the resting spore and the formation of what he calls a sexual oospore within the old sporangium. In 1892 Fischer combined the Rhizidiaceae and Polyphagaceae into the new family Sporochytriaceae and segregated all species of A. Braun's genus *Rhizidium* whose sporangia, rhizoids, and resting spores are endophytic or intracellular into the new genus *Entophlyctis*. He included the chytrid described by Schenk in this new genus and gave it the name *Entophlyctis intestina*. Schroeter

reestablished the family Rhizidiaceae in his classification of the Chytridiales in 1897, and retained Fischer's genus *Entophlyctis* but limited it to the endophytic species without an apophysis at the base of the sporangium. All endophytic species with an apophysis he segregated into the new genus *Diplophlyctis*, and changed the name of *E. intestinalis* to *Diplophlyctis intestinalis*. Neither Fischer nor Schroeter gives any original figures of the stages of the life history of this chytrid, and their classification is undoubtedly based on the descriptions and figures of Schenk, Zopf, and Dangeard.

Since the time of these observations of its occurrence in cells of European Characeae apparently little attention has been given to this fungus. So far I have been unable to find any reference in the literature to its occurrence in America, and I have made a comparative study of forms from thirteen host species and four genera of the Characeae to determine their specific identity. With the view of comparing the forms which I have observed with those described by Schenk, Zopf, and Dangeard I am giving a brief account of the life history of *D. intestinalis* as it occurs in cells of American Characeae. A more detailed study of cleavage, nuclear division, pathogenicity, and other problems connected with its life history is now being made.

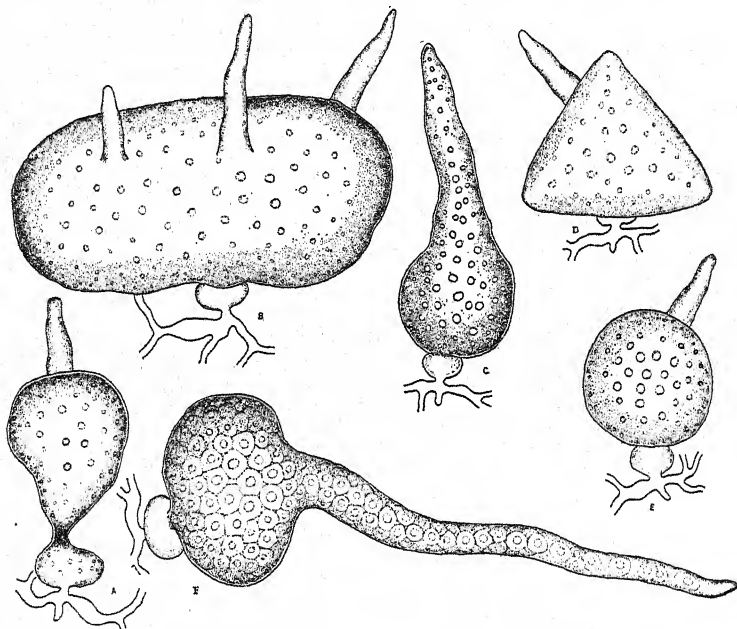
HABIT OF GROWTH AND GENERAL STRUCTURE

The mature form of *D. intestinalis* in the cells of the Characeae consists of a globular sporangium with an enlargement or swelling at the base, called the apophysis by Zopf; a sporangial neck, and a highly developed and richly branched rhizoidal or hyphal system which usually originates from the base of the apophysis. Text figure 1 shows a mature sporangium



TEXT FIG. 1. Habit of growth of *Diplophlyctis intestinalis* in dead cells of the Characeae.

with its neck, apophysis, and hyphal system. As is shown in this figure the sporangium usually lies in the center of a more or less radiating system of rhizoidal or hyphal filaments, and when it is observed under low magnification the appearance of the whole structure is very suggestive of a large single fungus spore which has germinated and produced a radiating and richly branched mycelium. The hyphal system is extensively developed, and as is shown in table 2 it may frequently have a radius of 380 microns. As Zopf pointed out in the form which he described it does not, however, direct itself in all directions and penetrate into the lumen of the cell but generally lies pressed against and spread on the inner periphery of the



TEXT FIG. 2. Variations in size and shape of the sporangia of *Diplophlyctis intestina* in cells of *Chara coronata*.

wall of the host cell. The main axis of the hyphal system arises from the base of the apophysis and forms from one to several main trunks which later divide into a large number of smaller branches. The branches usually direct themselves radially in a plane, perhaps by morphoesthesia as suggested by Hein (1927) for *Sphaerotheca*, thus producing a system of filaments more or less circular in outline. When only one or two trunks are formed from the base of the apophysis a one-sided system is developed whose contour or outline varies considerably from the circular.

The mature sporangia vary in size and shape in the same host cell. As is shown in text figure 2 of sporangia from a cell of *Chara coronata* they may be either pear-shaped (2 A), oval and flattened (2 B and Pl. XIV,

figs. 9, 10, 11), somewhat cylindrical and club-shaped (2 C), or spherical (2 E). Furthermore, it is not uncommon to find large sporangia which are quite irregular in outline. The diameter of the sporangia which I have observed varies from 8 microns in the small spherical ones to 30×88 microns in the large oval forms, while the largest sporangia reported by Zopf were 40 microns in diameter. With the view of determining the range of variation in size and shape of the sporangia and whether or not there may be more than one species of *Diplophlyctis* in these different hosts I have made, as noted, a comparative study of and measured the sporangia in cells of thirteen species of Characeae. In the first column of table 1 is shown the variation in size of the sporangia in seven species from four genera of the Characeae. The largest sporangium, measuring 30×88 microns, was found in a cell of *Nitella glomerulifera*. It is apparent from this table, however, that the range of variation, with this one exception, is very much the same in all hosts, and that as far as size and shape are concerned the sporangia in widely different hosts appear to be of the same general type. As noted before, *D. intestinalis* has been reported in cells of four species of *Nitella* and *Chara* by Schenk, Zopf, and Dangeard; and I have observed it in four species of *Nitella*, seven of *Chara*, and one each of *Lamprothamnus* and *Lychnothamnus*.

TABLE 1. Variation in Size of Sporangia, Zoöspores, and Resting Spores, and Character of the Wall of Resting Spores, in Seven Species from Four Genera of the Characeae

Host	Diameter of Sporangium	Diameter of Zoöspores	Diameter of Resting Spores	Wall of Resting Spores
<i>Chara coronata</i>	$12 \times 8 \mu$ to $50 \times 30 \mu$	4-6 μ	$10 \times 8 \mu$ to $20 \times 18 \mu$	Rough
<i>C. fragilis</i>	$10 \times 8 \mu$ to $40 \times 36 \mu$	4-6 μ	$10 \times 12 \mu$ to $20 \times 22 \mu$	Rough
<i>C. delicatula</i>	$8 \times 12 \mu$ to $30 \times 50 \mu$	4-6 μ	$10 \times 14 \mu$ to $22 \times 28 \mu$	Rough
<i>Nitella flexilis</i>	$12 \times 14 \mu$ to $28 \times 30 \mu$	4-6 μ	$8 \times 12 \mu$ to $18 \times 26 \mu$	Rough
<i>N. glomerulifera</i>	$10 \times 8 \mu$ to $30 \times 88 \mu$	4-6 μ	$8 \times 12 \mu$ to $24 \times 28 \mu$	Rough
<i>Lamprothamnus alopecuroides</i>	$10 \times 14 \mu$ to $28 \times 30 \mu$	4-6 μ	$12 \times 12 \mu$ to $22 \times 26 \mu$	Rough
<i>Lychnothamnus barbatus</i>	$8 \times 14 \mu$ to $25 \times 40 \mu$	4-6 μ	$10 \times 11 \mu$ to $22 \times 27 \mu$	Rough

The sporangial neck, as is shown in figures 8 and 9 by Schenk and in Plate XIV, figures 9, 10, 11, and 12, of my preparations, usually tapers from base to apex and varies considerably in length and diameter. If it develops on the periphery of the sporangium nearest the host wall and grows perpendicularly from the sporangium it may not be longer than the host wall is thick. The sporangial neck may often grow parallel with the wall of the host cell for a considerable distance before penetrating it, and in such cases it becomes very long. One sporangium with a diameter of

36 microns was found which had a sporangial neck 110 microns long. A somewhat similar sporangium is shown in text figure 2 *F*. The neck is not always formed on the periphery of the sporangium nearest the host wall, as is shown in figure 12 by Zopf, but may grow out from the opposite side and then curve around until it reaches the host wall. Oftentimes the sporangium and neck may be so continuous that it is difficult to differentiate one from the other (text fig. 2 *C*). This figure shows a sporangium which is somewhat spherical at the base and then tapers to a blunt point at the apex. The tapered end of the sporangium in such cases serves as the sporangial neck. Generally only one neck is formed on the periphery of the sporangium, but a few sporangia, such as the one shown in text figure 2 *B*, have been found with as many as three outlets for the escape of the zoöspores.

The apophysis generally lies at the base of the sporangium and is continuous with it up to the time of cleavage. When mature it is more or less oval, spherical, or somewhat unsymmetrical in shape. As the sporangium enlarges and comes into contact with it the apophysis may be flattened considerably, as is shown in text figures 2 *B*, 2 *F*, and Plate XIV, figures 9, 10, and 11. According to Zopf, it is continuous with the hyphal system throughout the entire life of the organism.

The cytoplasm of the mature sporangium is usually very granular in appearance with a large number of highly refractive bodies suspended or imbedded in it, as is shown in text figures 2 *A* and 2 *E*. Figure 11 shows a sporangium in which the process of cleavage is complete and the individual zoöspores have been delimited. Due to the mutual pressure on each other in the sporangium the zoöspores at this stage are more or less hexagonal in shape and generally contain one refractive body. When the sporangial neck is quite large, as is shown in text figures 2 *E* and 2 *F* and in Plate XIV, figure 11, its protoplasm undergoes cleavage, and it is not uncommon to find sporangia whose broad necks are full of mature spores. The number of zoöspores produced in a single sporangium varies to a considerable degree, depending largely on the size of the sporangium. Small sporangia with as few as two zoöspores have been observed, while larger ones similar to the sporangium shown in text figure 2 *B* have been found with more than 250 zoöspores.

As was early described by Schenk and Zopf for the European forms the zoöspores escape from the sporangium as soon as the apex of the neck ruptures. If the diameter of the neck is small the zoöspores elongate considerably as they pass out. As is shown in figure 12 several spores may be found in the neck at the same time. The flagellum always extends backwards from the spore body as it escapes, as in *E. heliomorpha*. According to Schenk and Zopf, the zoöspores become amoeboid in shape and method of movement as soon as they have escaped from the sporangium and before they have begun to swim about. They may also undergo

changes in shape as a result of their flagella being caught or held in the sporangial neck by the closely crowding zoöspores, as was described for *E. heliomorpha*. The flagella are very long, and the spore body may often be as far as 18 microns from the neck of the sporangium before its flagellum is entirely free. By careful focusing and close observation one can always see the fine hyaline flagellum connecting the body of the spore with the neck of the sporangium. Under such conditions where the flagellum is held or caught in the sporangial neck the zoöspores appear to pull and strain from side to side, swinging pendulum-like in a semicircle, form pseudopods, and undergo various contortions in attempting to free themselves. As soon as they are entirely free they tend to assume their ordinary spherical shape.

The zoöspores vary from 4 to 6 microns in diameter with a long flagellum attached at the posterior end. The flagellum may often be six times as long as the diameter of the spore body. As is characteristic of the zoöspores of the Rhizidiaceae, a highly refractive lobular body lies in the center or slightly displaced towards the posterior end of each zoöspore. Due apparently to its optical homogeneity the nucleus is usually invisible in living material but may sometimes be seen in the center of the spore. Plate XIV, figure 1, shows two zoöspores with refractive bodies and long flagella. In these respects they are identical with the zoöspores of *E. heliomorpha*. With the exception of perhaps a slight difference in size the zoöspores of both chytrids are so much alike that it is almost impossible to distinguish them when they occur in the same mount. This similarity also extends to their method of movement and swimming. When free from the sporangium they dart back and forth, often coming to an abrupt stop and then darting off again. As noted, the flagellum always extends backwards from the body of the spore as it swims about. As was described for *E. heliomorpha* in a previous paper (1928), the zoöspores show amoeboid movement and changes in shape if obstructed or trapped in a position where it is impossible for them to swim freely. After swimming about for from half an hour to two hours the zoöspores usually come to rest on the wall of the host cell and germinate. So far I have not observed their germination in a hanging drop apart from the host cell.

As was described by Schenk and Zopf, I find that the zoöspore generally forms a pointed germ tube which penetrates the wall of the host cell and forms a sporangium at its tip. This may often take place in less than three-quarters of an hour. Plate XIV, figure 2 A, shows a zoöspore which has germinated and whose germ tube has penetrated the wall of the host cell. As soon as the germ tube gets through the wall it begins to enlarge at its tip. This swelling or enlargement is the young sporangium. During germination the refractive body of the zoöspore usually moves down into the swollen end of the germ tube, as is shown in figure 2 A. In this figure may also be seen the beginning of the apophysis as a small papilla-like outgrowth from the enlarged tip of the germ tube.

A number of cases have been observed in which the germ tube did not penetrate completely through the wall of the host cell, but enlarged at its tip and formed a sporangium between the layers of the wall itself, as is shown in figure 4. Under such conditions the layers of the host wall are split farther and farther apart as the sporangium continues to grow in size.

As noted, the apophysis begins as a small papilla-like outgrowth at the base of the young sporangium. This papilla continues to elongate a few microns and then begins to enlarge. Very shortly a bud or pseudopod-like structure grows out from its base and branches from one to several times. This is the beginning of the rhizoidal or hyphal system. In the meantime, the sporangium has slowly enlarged, as is shown in figures 2 B, 3, and 5, but it does not attain its maximum rate of growth and size until the hyphal system is almost fully developed. Shortly after the appearance of the initials of the hyphal system the filaments begin to grow and develop very rapidly, so that a young and quite small sporangium may frequently be found with an extensively developed hyphal system. In table 2 is shown a comparison of the diameters of fifteen young sporangia and apophyses, and the radius of their hyphal system. As is shown in this table a young sporangium whose diameter is no more than 12×10 microns may have a hyphal system whose radius is as much as 380 microns.

TABLE 2. *Diameters of the Young Sporangium and Apophysis as Compared With the Radius of the Hyphal System*

Diameter of Sporangium	Radius of Hyphal System	Diameter of Apophysis
$14 \times 18 \mu$	200 μ	$4 \times 6 \mu$
$8 \times 18 \mu$	240 μ	$5 \times 6 \mu$
$8 \times 10 \mu$	320 μ	$5 \times 6 \mu$
$10 \times 12 \mu$	220 μ	$4 \times 6 \mu$
$10 \times 12 \mu$	380 μ	$5 \times 6 \mu$
$6 \times 12 \mu$	280 μ	$4 \times 4 \mu$
$10 \times 12 \mu$	220 μ	$4 \times 5 \mu$
$10 \times 10 \mu$	280 μ	$4 \times 6 \mu$
$8 \times 6 \mu$	200 μ	$3 \times 5 \mu$
$8 \times 10 \mu$	360 μ	$4 \times 6 \mu$
$10 \times 10 \mu$	300 μ	$6 \times 6 \mu$
$8 \times 8 \mu$	300 μ	$5 \times 6 \mu$
$6 \times 8 \mu$	160 μ	$3 \times 4 \mu$
$8 \times 5 \mu$	110 μ	$3 \times 4 \mu$
$10 \times 10 \mu$	300 μ	$5 \times 6 \mu$

As soon as the hyphal system is well formed the young sporangium begins to enlarge very rapidly. At first it is usually pear-shaped (figs. 3 and 5) and is not much larger in diameter than the apophysis. The connection between the two at this stage appears quite large in comparison with the size of the sporangium, but as it enlarges they come to lie close together. Figures 2 and 3 show the young sporangium, apophysis, and

hyphal system extending a considerable distance into the lumen of the host cell. Such cases appear to be exceptional, since the majority of sporangia appear to lie with their long axis parallel to and closely adhering to the inner periphery of the host cell wall. The hyphal system thus usually lies in the primordial utricle of the host cell and closely applied to the wall.

As the sporangium increases in size the number of refractive bodies increases, and for a time the cytoplasm appears highly vacuolated, as is shown in Plate XIV, figures 3, 4, 5, 6, and 7. Figure 6 shows a stage in development in which the sporangium has begun to increase in diameter and lose its pear-shaped appearance. A little later stage is shown in figure 7, in which the sporangium is almost spherical. The apophysis shown in this figure is quite large. The number of refractive bodies has increased and the cytoplasm appears less vacuolated. Figure 8 shows a later stage of development in which the sporangial neck has begun to appear on the surface of the sporangium. The sporangium shown in this figure has two necks, although, as noted before, usually only one is formed. The wall of the sporangium at this stage begins to thicken; the cytoplasm appears to become more granular and less vacuolated in appearance, and the number of refractive bodies increases.

In figure 9 is shown a somewhat small but almost mature sporangium which developed between the layers of the host cell wall. The layers of the wall have been pushed apart by the growth of the sporangium, and as a result of mutual pressure the sporangium itself has become somewhat flattened. The sporangial neck has begun to penetrate the upper layer of the host cell wall. In this particular case the hyphal system grew and spread between the layers of the wall for a considerable distance, but similar sporangia have been found whose hyphae penetrated the wall and then spread more or less radially in the primordial utricle of the host cell.

Up to the stage shown in figure 9 the fungus with its hyphal system, apophysis, sporangium, and sporangial neck is unicellular, but very shortly the sporangium and apophysis, according to Zopf, become separated by a wall. The presence of this wall is difficult to determine with certainty in living material because of the hyaline appearance of the whole structure. There appears to be no further development of the hyphal system after the sporangium has reached the stage shown in figures 9 and 10. Figure 10 shows a large mature sporangium shortly before cleavage. The neck is quite large in diameter and extends a considerable distance beyond the surface of the sporangium.

FORMATION OF THE RESTING SPORE

As noted before Zopf was the first to observe and figure the resting spore of *D. intestinalis*, and according to his description and my own observation it develops in the same host cell and in the same manner as the spo-

rangium. Dangeard (1890), on the other hand, has figured and described in addition to the ordinary resting spore an oöspore which he believes is the evidence of a primitive form of sexuality. According to Dangeard, in old cultures the protoplasm of the sporangium condenses into a central mass which becomes surrounded by small oil globules. A membrane or wall is then formed cutting off a central spore from a periplasm. The periplasm disappears little by little as it is used up in the formation of the numerous spines which cover the wall of the mature central spore. Dangeard did not observe antheridia, but described certain swellings on the oögonium which he believed might be vestigial antheridia. So far I have been unable to confirm Dangeard's observations as to the formation of an oöspore in the sporangium.

In the formation of the resting spore a sporangium-like structure with an apophysis and highly developed hyphal system develops at the tip of the germ tube after it has penetrated the wall of the host cell. After this structure, which is identical in appearance with the sporangium, has reached a certain age and size, ranging from 8 to 20 microns in diameter, its wall begins to thicken and the cytoplasm becomes coarsely granular in appearance. In figure 13 is shown an early stage in the formation of this resting spore. Figure 14 shows a slightly later stage. The spores shown in these figures are almost identical in appearance with the sporangium, with the exception that the wall is thicker and the cytoplasm more granular. In figure 14 the wall has become quite thick, and on its surface may be seen short stubby spines. These continue to develop as the wall grows thicker, until they become rather conspicuous in the mature spore. The apophysis and hyphal system is generally present with the spore for a considerable length of time after it has matured. Figures 15, 16, and 17 show mature resting spores of various sizes and shapes. Their content is coarsely granular and often appears as if it had undergone cleavage into zoöspores. As is shown in table I, the resting spores vary from 8×10 to 24×28 microns in diameter in individuals from the same host cell.

So far germination of the resting spores has not been observed in my cultures, although a large number have been kept under observation for a considerable length of time. Zopf does not figure any germination stages of the resting spores but states that the protoplast undergoes cleavage into spores and that a long neck or tube is formed from the resting spore which penetrates the wall of the host cell through which the zoöspores escape.

HOSTS AND PATHOGENICITY OF *DIPLOPHLYCTIS INTESTINA*

As noted before, *D. intestina* has been reported to occur in *Nitella flexilis*, *N. mucronata*, *N. tenuissima*, and *Chara polycantha* in Europe by Schenk, Zopf, and Dangeard. I have found it in cells of species of four genera of the Characeae including *Nitella flexilis*, *N. gracilis*, *N. tenuissima*,

N. glomerulifera, *Chara fragilis*, *C. zeylanica*, *C. disjuncta*, *C. coronata*, *C. vulgaris*, *C. contraria*, *C. delicatula*, *Lamprothamnus alopecuroides*, and *Lychnothamnus barbatus*. Whether or not the forms occurring in cells of all of these hosts belong to one species is not yet altogether certain, but they appear identical in their morphological characters and method of development. In 1889 Dangeard described and gave two figures of another species, *D. Catenatum*, in cells of *N. tenuissima*, which he believes differs from *D. intestina* in the shape of the sporangium and the presence of more than one enlargement or apophysis at the base or one side of the sporangium. I have often found sporangia and apophyses of what I take to be *D. intestina* which resemble in shape and appearance the sporangia of *D. Catenatum* as described by Schenk.

Since he could find *D. intestina* only in dead cells of *Nitella*, Zopf regarded it as a saprophyte or a weak parasite, and so far I have failed to find it in healthy green cells. In the light of the fact that it has been reported to occur only in dead cells it is perhaps not improbable that it will grow in dead cells of all species of Characeae. With the view of determining whether or not there are morphological and also physiological species of *Diplophlyctis*, I am at present attempting to infect a large number of species of Characeae with *D. intestina* from cells of *C. coronata*.

Like *Entophlyctis heliomorpha*, it is generally associated with a large number of parasites and saprophytes, such as *Diplophysalis* and *Vampyrella*, and it is perhaps possible that *D. intestina* gets into the host cell after the latter has been killed by these parasites. So far no intensive study of the degree of pathogenicity has been made on this group of Chytridiales, and at present I am attempting to determine the range of host plants and the degree of pathogenicity of *D. intestina*.

I feel much indebted to Prof. R. A. Harper for his generous and helpful criticism in this study.

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DESCRIPTION OF PLATE XIV

All figures were drawn from living material with the aid of a Spencer camera lucida and a Zeiss 2 mm. apochromatic objective N.A. 1.30, and compensating ocular number 8.

FIG. 1. Two zoöspores showing the highly refractive body and long flagellum attached at the posterior end. One of the zoöspores is somewhat elongated and has formed short pseudopods.

FIG. 2 A. A germinated zoöspore whose germ tube has penetrated the wall of the host cell and begun to enlarge at its tip. The refractive body of the spore has migrated down the germ tube and lies in the enlarged tip.

FIG. 2 B. An early stage in the development of the sporangium. The apophysis appears as a small swelling at the base of the sporangium.

FIG. 3. A young pear-shaped sporangium with a fairly well developed apophysis.

FIG. 4. A small sporangium which has begun to develop between the layers of the host cell wall.

FIG. 5. A small pear-shaped sporangium with the initials of the hyphal system well developed.

FIG. 6. A later stage in the development of the sporangium. The sporangium has become more spherical in shape and the apophysis has enlarged.

FIG. 7. A slightly later stage in the formation of the sporangium.

FIG. 8. A still later stage in the development of the sporangium. Two sporangial necks have begun to form. The apophysis is comparatively small.

FIG. 9. An almost mature flattened sporangium which has developed between the layers of the host cell wall.

FIG. 10. A large, somewhat flattened sporangium shortly before cleavage.

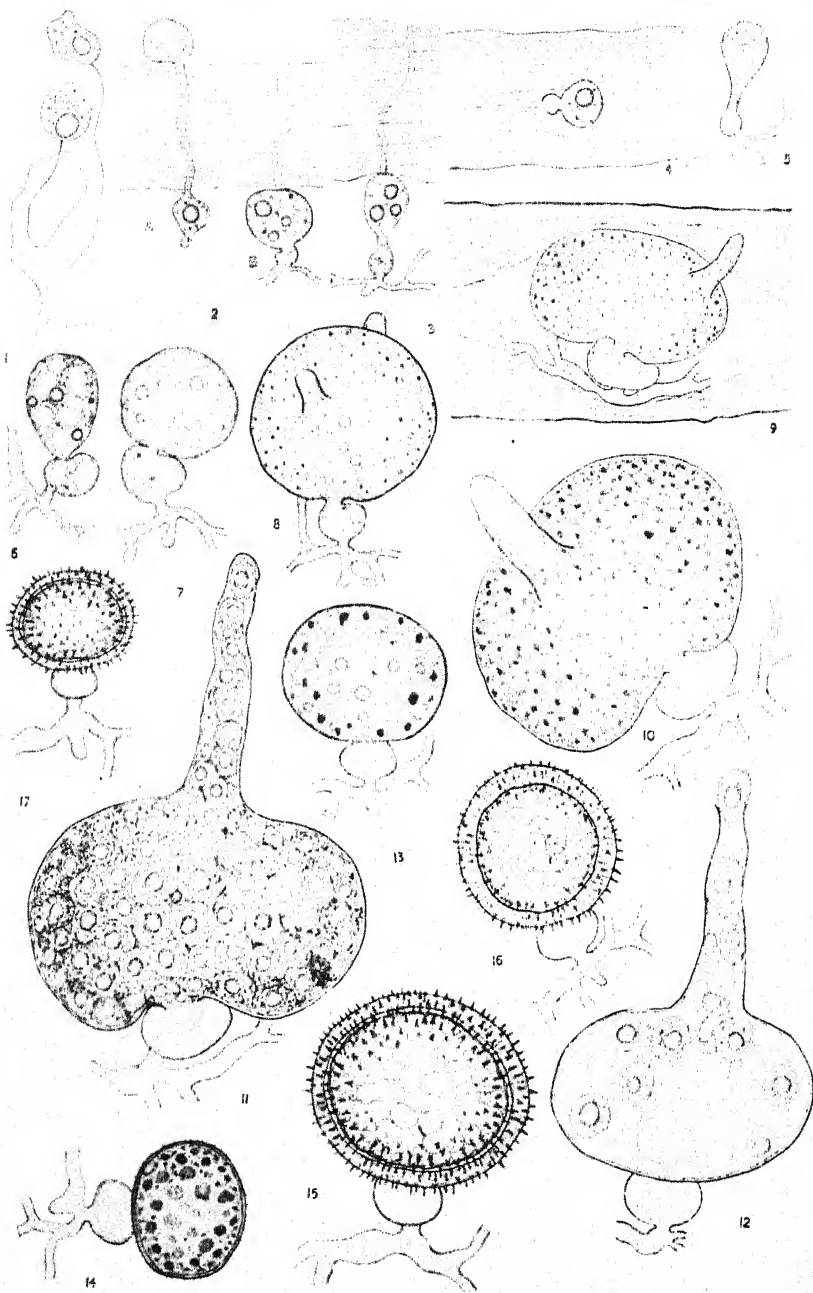
FIG. 11. A large sporangium which has undergone cleavage into zoöspores.

FIG. 12. A large mature sporangium with escaping zoöspores.

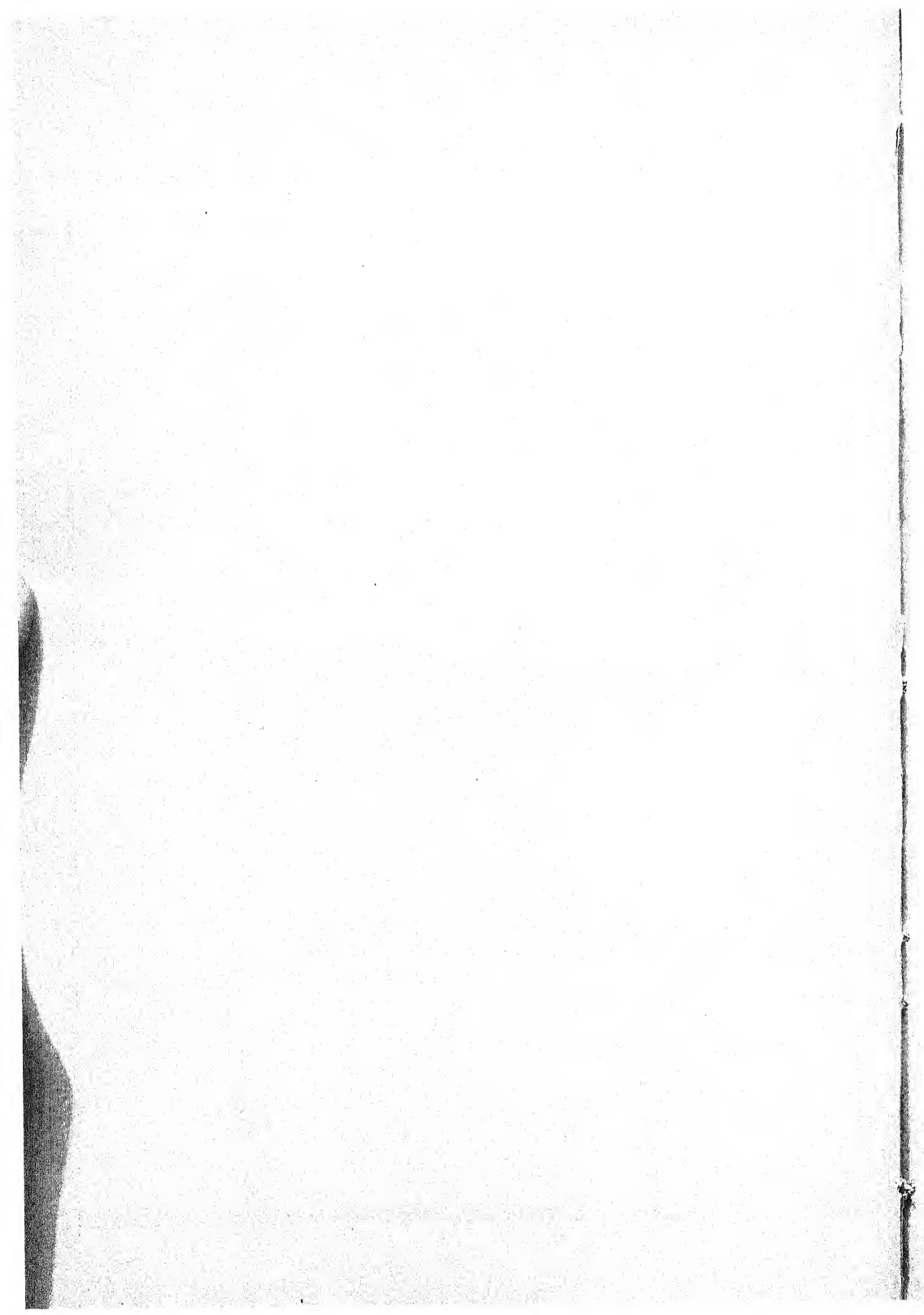
FIG. 13. An early stage in the formation of the resting spore. The wall of the young spore has begun to thicken, and the cytoplasm has become coarsely granular in appearance.

FIG. 14. A later stage in the formation of the resting spore. Small spines have begun to appear on the surface of the spore.

FIGS. 15, 16, and 17. Mature resting spores of various sizes and shapes.



KARLING: DIPLOPHLYCTIS



THE AUSTRALASIAN ELEMENT IN THE HAWAIIAN FLORA

DOUGLAS HOUGHTON CAMPBELL

(Received for publication August 25, 1927)

The flora of the Hawaiian Islands, as is well known, is a highly specialized one, a very large percentage of the species being peculiar to the archipelago. This remarkable degree of endemism is, of course, due to the extreme isolation of the Islands.

The origin of the existing flora has been the subject of a good deal of speculation, and there is a wide difference of opinion as to how plants have reached this remote region.

The most generally accepted theory of the origin of the archipelago is that the islands have been built up by volcanic action from a deep-seated submarine ridge, and have always been completely isolated and far removed from any land mass of considerable size. This necessarily implies that the present rich indigenous flora has been introduced from outside since the emergence of the islands.

Opposed to this view is the theory that the archipelago, as it now exists, is but a remnant of a much larger land-mass which has been in subsidence for a very long period, and that very extensive subsidence has also occurred throughout Polynesia, and to a lesser extent in Australasia. Among the reasons for this assumption is the widespread development of coral-reefs in the Pacific, especially in Polynesia and northeastern Australia. The existence of active coral-reefs involves continuous subsidence,¹ and the absence of large land-masses in mid-Pacific, with the innumerable small islands and reefs, can be explained most satisfactorily on the assumption that these coral islands and reefs are the remnants of a sunken continent, or continental islands.

The floor of the great Pacific basin is still very imperfectly explored; but an examination of such maps as have been published² reveals some very interesting facts concerning the relation of the Hawaiian Archipelago to the regions of the southern Pacific.

Between Hawaii and North America, and extending almost completely round the islands, is an enormous area of extremely deep water, the Tuscarora and Belknap Deep. To the west, however, is a much shallower area which connects with the relatively shallow seas of Polynesia and Australasia, between which and Hawaii are no considerable areas comparable with the great deeps separating Hawaii from America. It is possible,

¹ Davis, W. M. The depth of coral-reef lagoons. Proc. Nat. Acad. Sci. 9: 1923.

² E.g., Century Atlas, Map 2.

therefore, that the western limits of the great deeps may indicate the position of the margin of a sunken continental area—or areas—now occupied by the shallower seas extending from Hawaii through Polynesia to Malaya and Australasia. The overwhelming preponderance of Malayan and Australasian types in the Hawaiian flora can only be accounted for satisfactorily on the assumption of some much more intimate connection than now exists between the archipelago and the lands of the South Pacific.³

A large part of the Hawaiian flora consists of species which show no indication of adaptation for transport over such immense expanses of sea as separate Hawaii from the South Pacific lands, where their nearest relatives are found; and no conceivable agencies exist which could effect such transport.

An analysis of the Hawaiian flora indicates that on the whole the relationships are mainly Malayan and Australasian, although a good many of the common genera are widespread in the Old World, and others are cosmopolitan. The most significant fact is the large number of genera quite absent from America. As examples of these extra-American genera may be mentioned *Pandanus*, *Freycinetia*, *Dracaena*, *Santalum*, *Viscum*, *Gardenia*, *Achyranthes*, *Alphitonia*, *Alyxia*, *Cyrtandra*, *Antidesma*, and many others.

As North America is the continent nearest to Hawaii, and there are in active operation the various agencies by which it is assumed plants have been introduced—*viz.*, migratory birds, northeast trade winds, ocean currents from the west coast to the islands—one might expect a marked preponderance of American types in the flora, but such is far from being the case. It is true that there is a considerable number of genera of undoubted American origin, like *Sisyrinchium*, *Raillardia*, and others; and the introduction of these by migratory birds, wind, or—less probably—water transport, may very well be taken for granted; but the number of these American immigrants is surprisingly small when compared with the number of species unmistakably allied to those of the Malayan and Australasian regions, at present absolutely cut off from Hawaii and with no conceivable means by which these plants could have been transported over the vast expanse of ocean lying between.

While Australia and New Zealand share with the Malayan regions many genera and a good many species, there are certain genera, and even families, which are mainly or exclusively confined to Australasia; and it is this element of the Hawaiian flora to which attention is here directed.

In this connection I shall consider both Australia and New Zealand as belonging to Australasia, although New Zealand has less in common with Australia than is often supposed and might very well be separated as a distinct botanical province.

³ Campbell, D. H. The derivation of the flora of Hawaii. Leland Stanford Junior University Publications, 1919.

An analysis of the Australasian floras shows three fairly distinct categories. In eastern and especially northeastern Australia, and throughout New Zealand, the vegetation is markedly Malayan in type, and there is abundant evidence that both countries had former connections with the Malayan regions lying to the north, from which were derived, probably independently, many related or identical species. It is these Malayan types which constitute the greatest similarities in the floras of the two countries.

The second element is the very large and peculiar autochthonous flora of Australia, probably originating in West Australia and scantily represented outside Australia. This peculiar flora has but a comparatively scanty representation in New Zealand. The latter has also a considerable number of characteristic genera (some endemic), and a large number of endemic species.

Finally, especially in New Zealand, there is a considerable number of species which are closely related to, or identical with, South American types. This "sub-antarctic" or "Fuegian" flora is found to some extent also in the cooler and moister regions of southeastern Australia, especially Tasmania and the mountains of Victoria and New South Wales, and has some representatives also in Hawaii, e.g., *Astelia*, *Nertera*, *Oreobolus*.

The following list of Hawaiian genera may with some considerable degree of probability be assumed to be more or less directly related to Australasian rather than Malayan types, although most of them have representatives outside Australia and New Zealand.⁴

<i>Acacia</i> (Leguminosae)	<i>Geranium</i> (Geraniaceae)
<i>Acaena</i> (Rosaceae)	<i>Lagenophora</i> (Compositae)
<i>Alphitonia</i> (Rhamnaceae)	<i>Metrosideros</i> (Myrtaceae)
<i>Alyxia</i> (Apocynaceae)	<i>Myoporum</i> (Myoporaceae)
<i>Astelia</i> (Liliaceae)	<i>Myrsine</i> (Myrsinaceae)
<i>Baumea</i> (Cyperaceae)	<i>Nertera</i> (Rubiaceae)
<i>Byronia</i> (Rhamnaceae)	<i>Oreobolus</i> (Cyperaceae)
<i>Cassytha</i> (Lauraceae)	<i>Ochrosia</i> (Apocynaceae)
<i>Coprosma</i> (Rubiaceae)	<i>Pittosporum</i> (Pittosporaceae)
<i>Cordyline</i> (Liliaceae)	<i>Portulaca</i> (Portulacaceae)
<i>Cyathodes</i> (Epacridaceae)	<i>Pseudomorus</i> (Urticaceae)
<i>Dianella</i> (Liliaceae)	<i>Santalum</i> (Santalaceae)
<i>Dodonaea</i> (Sapindaceae)	<i>Scaevola</i> (Goodeniaceae)
<i>Elaeocarpus</i> (Elaeocarpaceae)	<i>Sophora</i> (§ <i>Edwardsia</i>) (Leguminosae)
<i>Exocarpus</i> (Santalaceae)	<i>Vittadinia</i> (Compositae)
<i>Gahnia</i> (Cyperaceae)	<i>Wikstroemia</i> (Thymeliaceae)

⁴ In preparing this list the following works were consulted: Hillebrand, "Flora of the Hawaiian Islands"; Engler and Prantl, "Die Natürlichen Pflanzenfamilien"; Kew Index; Cheeseman, "Manual of the New Zealand Flora"; Bentham, "Flora Australiensis."

While it seems most likely that all of the genera cited above are predominantly Australasian, they have representatives widespread in Malaya, in some cases extending to the Asiatic mainland and even across the Indian Ocean to Madagascar and the Mascarene Islands. It might therefore be questioned whether these genera should not be regarded as Malayan rather than Australasian. The following genera fall into this category: *Alphitonia*, *Alyxia*, *Elaeocarpus*, *Cordyline*, *Wikstroemia*.

The following genera are predominantly Australian, and in some cases almost confined to the Australian continent: *Acacia*, *Byronia*, *Cassytha*, *Cyathodes*, *Dianella*, *Dodonaea*, *Exocarpus*, *Gahnia*, *Myoporum*, *Pseudomorus*, *Santalum*, *Scaevola*, *Vittadinia*.

Acacia is, of course, almost cosmopolitan, but it is in Australia, with about 450 species, that it reaches its greatest development; and only in Australia and Polynesia do the phyllodineous species occur. All the three indigenous Hawaiian species, including the well-known Koa, belong to this section of the genus.

Byronia is a genus of three species confined to Australia, Tahiti, and Hawaii.

Cassytha filiformis is a curious leafless parasite, suggesting *Cuscuta*, but a member of the laurel family. It is widespread through the Old World tropics, and reaches Hawaii. Aside from this species, however, together with a single one in Borneo and two in South Africa, the others (about a dozen) are confined to Australia.

Cyathodes, belonging to the almost exclusively Australian family Epacridaceae, is represented in Hawaii by two species, one of which, *C. Tameiameiae*, also occurs in Tahiti. Otherwise, the genus is confined to Australia and New Zealand.

The liliaceous *Dianella* has one widespread species, *D. ensifolia*, but of the eleven species (Engler and Prantl) eight occur in Australia.

Like *Dianella*, *Dodonaea* has one widespread species, even reaching the New World; but forty-four out of the forty-six species are Australian. Hawaii (Hillebrand) has three species, the widespread *D. viscosa* and two endemic.

Exocarpus, with ten out of fourteen species confined to Australia, has two endemic species in Hawaii.

Gahnia, a characteristic genus of Australasian sedges with about thirty species, is represented in Hawaii by five. One of these, *G. Gaudechaudii*, is also found in New Zealand. Australia has about half the total number of species, and New Zealand about one-fourth. The remainder are scattered through Polynesia and tropical Asia.

Myoporum, of the almost exclusively Southern Hemisphere family Myoporaceae, is predominantly Australian, a large majority of the species being confined to Australia. The single Hawaiian species is endemic.

The monotypic *Pseudomorus Brunoniana* is confined to Australia, Norfolk Island, New Caledonia, and Hawaii.

Santalum comprises a small number (about eight or nine) of closely related small trees or shrubs, some of which yield the sandalwood of commerce. Species occur in the Asiatic tropics and in Polynesia, but the majority are confined to Australia and Hawaii—each with three species, those of Hawaii being all endemic.

The genus *Scaevola* affords one of the most convincing examples of the Australian element in the Hawaiian flora. *Scaevola* belongs to a family, Goodeniaceae, which is developed to a remarkable degree in Australia but is very poorly represented elsewhere. A very few species, e.g., *S. Lobelia* L., are widespread as strand plants in the tropics, the latter even reaching the West Indies; but, according to Hillebrand, outside of Hawaii, which has seven endemic mountain species, a single species from New Caledonia is the only other known from Polynesia, while Australia has over fifty species.

Vittadinia, according to Engler and Prantl, has seven species, four in Australia, two in New Guinea, and a single one in southern Brazil. The Kew Index unites the endemic Hawaiian genus *Tetramolopium*, comprising seven species, with *Vittadinia*, and recognizes five Australian and two South American species.

Whether or not *Tetramolopium* is recognized as a distinct genus, it is obvious that the Hawaiian species are closely related to the Australian *Vittadinia*.

In addition to the foregoing genera, two others, *Portulaca* and *Geranium*, may possibly have their nearest relationships with Australian species. Hawaii has six endemic species of *Geranium* and two of *Portulaca*. Of course *Geranium* is a cosmopolitan temperate genus and may have been derived from America; but while *Portulaca* is a characteristic South American genus, it is most likely that the Hawaiian species are more nearly related to those of northern Australia, where there are six endemic species.

The relationships of the Hawaiian flora with that of New Zealand are unmistakable. The following characteristic New Zealand genera are well represented in Hawaii: *Acaena*, *Astelia*, *Baumea*, *Coprosma*, *Gunnera*, *Metrosideros*, *Myrsine*, *Nertera*, *Oreobolus*, *Pittosporum*, *Sophora* (section Edwardsia).

Acaena is a genus mostly confined to the south temperate and subantarctic regions. Two species, however, extend to the mountains of Mexico and California. Six species belong to New Zealand, and three to Australia. The single Hawaiian species is endemic. It is possible, of course, that this might have reached Hawaii from America.

Astelia is a small liliaceous genus whose headquarters are in New Zealand. Species also occur in the mountains of southern Australia and Tasmania, and in the mountains of Fiji and Tahiti. There are two endemic Hawaiian

species. *Astelia* is one of the genera which New Zealand shares with sub-antarctic South America.

Baumea, a genus of sedges, sometimes united with *Cladium*, has about twenty species, mostly Australasian, but with outlying species in Polynesia and the islands of the Indian Ocean.

Coprosma, familiar to Californians through the extensively cultivated *C. Baueri*, is especially interesting, since of the (about) forty species twenty-seven are found in New Zealand and nine in Hawaii. The others are scattered in Australia, Fiji, Tahiti, and Borneo, and a single species occurs in Juan Fernandez.

Among the most remarkable plants of Hawaii is *Gunnera petaloidea*, found in the exceedingly wet mountain forests, where the enormous leaves at once attract attention and recall the Chilean species occasionally seen in cultivation.

The genus reaches its greatest development in Andean and sub-antarctic South America and New Zealand, the greatest number of species belonging to the latter. A single species is found in Java and Luzon, and it also is represented in South Africa and Costa Rica. It may be that the Hawaiian species is more nearly related to the Malayan one than to those of New Zealand.

Perhaps the commonest tree of Hawaii is *Metrosideros polymorpha*, the "Lehua" of the natives. This species is widespread in Polynesia, and is extremely variable. Two other endemic species are also known. New Zealand is headquarters for the genus with more than two-thirds of the known species, while Hawaii, with three species, is next. A single species is found in Australia, and one each in South Africa and the Malay Archipelago.

Among the common plants of the mountain forests of Hawaii is a pretty little trailing plant with scarlet berries, *Nertera depressa*. The geographical distribution of this species is very remarkable, as it ranges along the whole Andean chain as far north as Mexico, and south to Fuegia. It is common in New Zealand and Australia, and is reported from the mountains of Tahiti as well as Hawaii. A similar—perhaps identical—species is common in the higher mountains of the Malay Archipelago as far north as Luzon, in the Philippines. Four of the six species of *Nertera* are found in New Zealand, which would appear to be the headquarters of the genus.

Oreobolus is a small genus of sedges which, except for a single species in Hawaii, is confined to the mountains of New Zealand, Australia, and sub-antarctic South America.

The family Pittosporaceae is a small one, all the genera except *Pittosporum* being restricted to Australia. *Pittosporum*, however, with about seventy species, has a wide distribution, being found from tropical Africa and the Canaries to Australasia, Polynesia, and continental Asia as far

as China and Japan. The majority of the species, however, are Australasian, and the genus is especially developed in New Zealand. Hawaii, with ten endemic species, comes next to New Zealand in the number of species.

Sophora is a genus widely distributed in both the Old and the New Worlds. One section of the genus, *Edwardsia*, is especially characteristic of Andean South America and New Zealand. *S. tetraptera* of New Zealand is said to be identical with a Chilean species. It is to *Edwardsia* that the endemic Hawaiian *S. chrysophylla* belongs.

As might be expected from the extreme isolation of the islands, not only is the number of endemic species very large but there are also a good many peculiar genera. Among these the following may be cited as showing more or less evident relationships with Australasian genera:

Hawaiian	Australasian
<i>Brighamia</i> (Lobeliaceae)	<i>Isotoma</i>
<i>Tetramolopium</i> (Compositae)	<i>Vittadinia</i>
<i>Pelea</i> (Rutaceae)	<i>Melicope</i>
<i>Cheirodendron</i> (Araliaceae)	<i>Pseudopanax</i>

In the Kew Index *Pelea* is united with *Melicope*, and although the great majority of the species are Hawaiian, a single species occurs in Madagascar and another in New Caledonia.

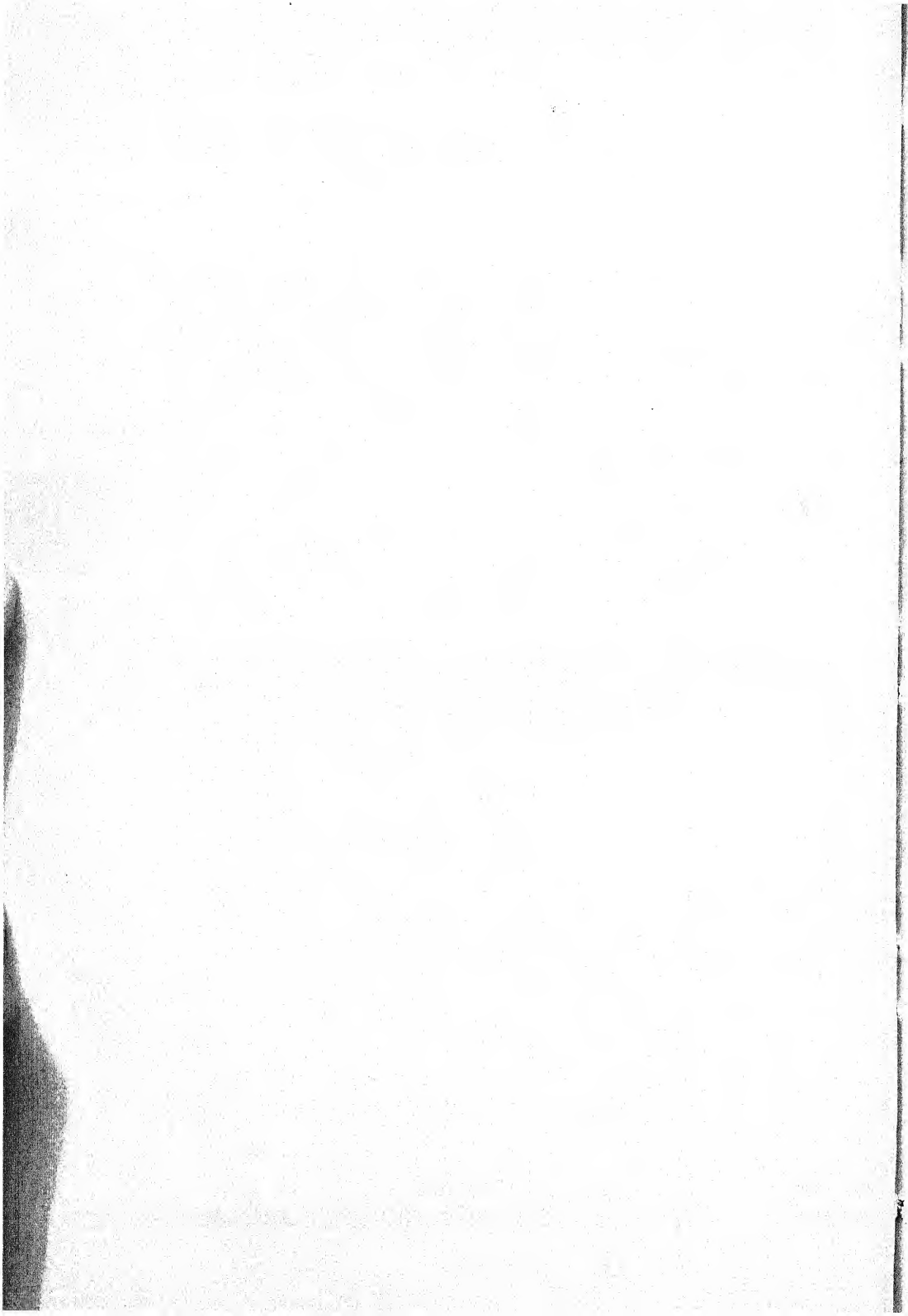
The Araliaceae of Hawaii are interesting and show certain parallelisms with those of New Zealand. Of the five genera recognized by Hillebrand, three are endemic, and of the other two, *Reynoldsia* is confined to Polynesia, and *Tetraplasandra* with two endemic Hawaiian species has a third species in New Guinea and the Sunda Islands.

Of the endemic genera, *Cheirodendron* is considered to be closely related to the New Zealand *Pseudopanax*.

Hawaii has five genera and twelve species of Araliaceae, compared with six genera and seventeen species in New Zealand; while the whole United States has but three genera and eight species.

CONCLUSIONS

From the data given in the foregoing pages, it is reasonable to conclude that there is a very real relationship between the floras of New Zealand and Australia, on the one hand, and that of the Hawaiian Archipelago on the other. Perhaps, on the whole, the relationships with New Zealand are somewhat more pronounced than those with Australia. On the basis of these evident relationships we are justified in assuming the former existence of land-masses of considerable size, connecting more or less directly both Australia and New Zealand with Hawaii.



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STUDIES IN ONAGRACEAE I. A REVISION OF THE SUB- GENUS CHYLISMIA OF THE GENUS *OENOTHERA*

PHILIP A. MUNZ

(Received for publication September 12, 1927)

In presenting this paper I am offering the first of a series of studies on which I have been working for the past three years and which are directed toward a monograph of the genus *Oenothera*, *sens lat.* As the work has progressed, the feeling has grown on me that most of the various segregate-genera are best kept together in a rather inclusive group. A discussion of the whole situation is reserved for a later date.

For this particular paper some seven hundred sheets have been available, including the material in the herbaria listed below. These herbaria were either visited or the material was borrowed. The abbreviation given with each name is that used in citing specimens:

Gray Herbarium (G)
New York Botanical Garden (NY)
Philadelphia Academy of Sciences (Ph)
University of California (C)
Stanford University (S)
Frank Peirson Herbarium (FP)
University of Wyoming (W)
Washington State College (WS)
National Herbarium (US), only some special material borrowed
Pomona College (P)

To those in charge of these herbaria, and particularly to Dr. B. L. Robinson of the Gray Herbarium for his kindness to me while working there, I express my appreciation of many courtesies shown.

The subgenus *Chylismia* is almost confined to the Great Basin region of western North America, extending from the Rocky Mountains to the Sierra Nevada and from Oregon and Idaho to Sonora and Lower California. Its closest relative is apparently the subgenus *Sphaerostigma* (over which name *Chamissonia* has priority), differing from the latter mostly in the

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pediceled capsules which are moreover quite free from contortion or looping. The species most closely allied to those in *Chamissonia* by the shortness of the pedicels are apparently *O. cardiophylla* and *O. brevipes*, particularly the latter with its cylindrical capsules. From *O. brevipes* one can trace two general lines of descent, both having smaller flowers; one goes to *O. clavaeformis* with its shorter capsules, basal leaves and simple stems, and the other to *O. multijuga* with branching habit, more evenly distributed leaves, and slender elongate capsules. *O. scapoidea* is a close relative of *O. clavaeformis*, having smaller flowers and less congested inflorescence. *O. heterochroma* and *O. Parryi* are near to *O. multijuga*, retaining the branching habit but having shorter capsules. *O. pterosperma* with its winged seeds and axillary flowers is quite remote from any other species in the subgenus and may have its affinity elsewhere.

SUBGENUS CHYLISMIA (Nutt.) Jepson, Man. Calif., 680. 1925

Section *Chylismia* Nutt., ex T. & G., Fl. N. Am. 1: 506. 1838-1840. S. Watson, Proc. Am. Acad. 8: 594. 1873. Genus *Chylismia* (Nutt.) Raimann in Engl. & Prantl, Nat. Pfl. Fam. 3: Abt. 7, 217. 1893.

Calyx-tube obconic to short funnelform. Stamens generally of two lengths, all fertile. Stigma capitate. Capsules clavate or cylindrical, not contorted, on well-defined pedicels. Usually annuals, with entire to pinnatifid leaves, these largely basal. Flowers white to yellow, often reddish in age.

KEY TO SECTIONS

- A. "Flowers axillary, very small; seeds oblong, deeply concave on the inner side and somewhat wing-margined, cellularly papillose." (Flores axillares minuti; semina oblonga, profunde concava intra, et alata.) *Chylismiella* n. sect.
1 species, *O. pterosperma*.
- AA. Flowers in terminal racemes; seeds obovoid, rounded or angled, not winged. (Flores in racemis terminalibus; semina obovoidea, rotundata aut angulata, inalata.) *Euchylismia* n. sect.
7 species: *O. cardiophylla*, *O. brevipes*, *O. multijuga*, *O. scapoidea*, *O. clavaeformis*, *O. heterochroma*, and *O. Parryi*.

KEY TO SPECIES

- Seeds oblong and with an incurving wing, making them appear somewhat boat-shaped, cellular-pubescent; small slender plants, villous below, finely glandular-pubescent above, with pinkish-white axillary flowers 4-5 mm. across. Ranging from Utah west to eastern Oregon and Inyo Co., California. 1. *O. pterosperma*.
- Seeds obovoid, rounded or angled, not winged; flowers not axillary, but in terminal racemes or panicles.
- Leaves orbicular-cordate, well distributed, not at all pinnatifid, commonly glandular-pubescent; plants rather coarse; flowers yellow, becoming bright red in age; anthers glabrous; capsules on rather short pedicels, and coarse-cylindrical. Inyo Co., California south into Lower California. 2. *O. cardiophylla*.
- Leaves ovate, oblong, or lanceolate, commonly pinnatifid and generally near base of plant.
- Capsules linear, elongate, usually over 2 cm. long.

- Stems coarse, commonly branched only at base; pedicels short, usually 3-15 mm. long; capsules linear, widely spreading, commonly 5-9 cm. long; anthers hairy.....3. *O. brevipes*.
- Stems slender, commonly freely branched above; pedicels capillary, 10-25 mm. long; capsules linear, 1.5-3.5 cm. long; anthers glabrous (except in var. *typica*).....4. *O. multijuga*.
- Capsules somewhat clavate, usually less than 2 cm. long.
- Branches in well-developed plants few to several and arising at base of plant only, not capillary; capsules 10-25 mm. long; anthers linear, beset with scattering white hairs; style longer than petals.
- Stems slender; flowers few, not congested; leaves ovate, subentire; petals (except in var. *Eastwoodae*) usually less than 4 mm. long. Wyoming and Colorado to Utah and eastern Nevada.....5. *O. scapoidea*.
- Stems fairly coarse; flowers crowded in close terminal clusters; leaves frequently with supplementary pinnules on petioles; petals 4-7 mm long. Eastern Oregon and Nevada to California and Arizona.
6. *O. clavaeformis*.
- Branches in well-developed plants capillary and arising freely throughout the plant; anthers oblong to linear-oblong, glabrous; style not longer than petals.
- Capsules 8-12 mm. long; anthers oblong, at least two-thirds as wide as long; style pubescent at base. Southwestern Nevada to Inyo Co., California.
7. *O. heterochroma*.
- Capsules 3-9 mm. long; anthers not over one-third as wide as long; style glabrous. Southern Utah.....8. *O. Parryi*.

TREATMENT OF SPECIES

I. OENOTHERA PTEROSPERMA S. Wats., Bot. King, 112. Pl. 14. 1871

Oenothera pterosperma S. Wats., Proc. Am. Acad. 8: 595. 1873; Leveille, Mon. Onoth., 149. 1905. *Chylismia pterosperma* (S. Wats.) Small, Bull. Torr. Club 23: 193. 1896. *Sphaerostigma pterospermum* (S. Wats.) A. Nels., Bot. Gaz. 40: 63. 1905.

Annual, low, 5 to 12 cm. tall, erect, simple or with few open branches; stem slender, with fine pilose hairs below, finely glandular above; leaves oblong- to ovate-lanceolate, acute to obtuse, often with a "shoulder" on each side of tip, entire, sessile to short-petioled, 5-20 mm. long, 2-5 wide, glabrate to finely pilose-pubescent; flowers axillary "pinkish-white"; pedicels 5-8 mm. long, capillary; calyx-tube obconic; ca. 1.25 mm. long, glabrous within; calyx-lobes lance-ovate, 1.7 to 2.5 mm. long; petals obcordate, equaling calyx-lobes; longer stamens about half the length of the petals, shorter one-fourth the length; pistil equaling corolla, stigma 0.7 mm. broad; capsules "cylindrical-clavate," slightly curved, membranous, erect, 10-16 mm. long, attenuate at base; seeds oblong, 1.5 mm. long, brownish, flattened, bordered with a revolute, winglike margin which is "minutely tubercled with cellular processes."

Type locality in the foothills of the Trinity Mts., northwestern Nevada. Material studied: UTAH: Southern Utah, Parry 67 (G), 70 (G); Gold Hill, Jones in 1891 (C, P); Minersville, Stokes in 1903 (C); Milford, Jones in 1880 (P); Frisco, Jones in 1880 (NY, P). NEVADA: Furber,

Jones in 1891 (P); Good Springs, *Jones in 1905* (C, P); Reno, *Hillman in 1895* (P); Candelaria, *Shockley 336* (G); Trinity Mts., *Watson 424*, type (G). OREGON: Harper Ranch, eastern Oregon, *Leiberg 2075* (C, G, NY). CALIFORNIA: west of Bishop, *Heller 8276* (G, NY, S).

In spite of the very different seeds this plant is quite like other smaller plants of the subgenus in superficial appearance; for example like *O. scaepoidea* or reduced forms of *O. multijuga* var. *orientalis*, and seems quite *Chylismia*-like. I cannot understand Leveille's (l. c.) citing *Kneiffia chrysantha* Spach and *K. pumila* Spach as synonyms of *O. pterosperma* unless it was due to careless reading of Watson (Proc. Am. Acad. 8: 611-612. 1873) where references and synonyms of *O. pumila* are cited immediately after the references for *O. pterosperma*.

2. OENOTHERA CARDIOPHYLLA Torrey, Pac. R. R. Rept. 5: 360. 1856

Annual to suffrutescent perennial, 1-5 (7) dm. high, erect, usually freely branched, occasionally simple, typically soft-pubescent throughout, but varying from glabrate to white-villous; stems fairly coarse; leaves orbicular-cordate to ovate and irregularly dentate or denticulate, obtuse, somewhat bicolored, subglabrate to white villous, well distributed; leaf-blades 10-60 mm. long and 8-50 wide; petioles 10-70 long; flowers borne singly in axils of reduced upper leaves or mostly in dense terminal racemes which are commonly ca. 10 cm. long; calyx-tube finely pubescent within, obconic, 5-35 mm. long; calyx-lobes ovate, 3-20 mm. long, with or without free subulate tips; petals a clear yellow, turning red with age, broader than long, 3-20 mm. long, rounded obovate; stamens glabrous, the longer filaments two-thirds as long as the petals, shorter ones ca. half as long as petals; style slightly exceeding petals, finely pubescent at base; stigma ca. 1 mm. across; capsules rather coarse, cylindrical, usually slightly curved, 20-60 mm. long, on pedicels 2-10 mm. long; seeds obovoid, brown, somewhat irregularly angled, ca. 0.6 mm. long.

KEY TO VARIETIES

Calyx-tube 5-10 mm. long; petals 6 (2)-8 mm.; style 12-16 mm. long.

2a. *O. cardiophylla* var. *typica*.

Calyx-tube 20-35 mm. long; petals 13-25 mm.; style 30-60 mm. long.

2b. *O. cardiophylla* var. *splendens*.

2a. *Oenothera cardiophylla* Torr. var. *typica* n. nom.

Oenothera cardiophylla Torr., l. c.; Leveille, Monogr. Onoth., 151 & pl., 1905. *Chylismia cardiophylla* (Torr.) Small, Bull. Torr. Club 23: 193. 1896. *O. cardiophylla* var. *petiolaris* Jones, Proc. Calif. Acad. ser. 2, 5: 682. 1895. *O. cedrosensis* Greene, Bull. Calif. Acad. 1: 187. 1885.

Calyx-tube 5(3)-10 mm. long; petals 6(2)-8 mm.; style 12-16 mm. long.

Type locality near Yuma, Arizona. Representative material: CALIFORNIA: Panamint Canyon, Inyo Co., *Hall & Chandler 7000* (C, P); Shepherds Canyon, *Jones in 1897* (P); Surprise Canyon, *Coville & Funston*

725 (G, NY, S); Mohave Desert, *J. G. Lemmon & wife, in 1884* (C); Painted Canyon, Riverside Co., *McGregor 736* (S), *Munz & Keck 9351* (P); Palm Canyon, *Johnston 1083* (P, S); Palm Springs, *Parish 4118* (C, G, NY); San Felipe Wash, San Diego Co., *Eastwood 2706* (G, NY); Borregos Springs, *K. Brandegee in 1899* (C); Signal Mt., Imperial Co., *Abrams 3167* (G, NY, S); Split Mt. Canyon, *Gilman in 1926* (P); Pilot Knob, *Jones in 1926* (P); Ft. Yuma, *Major Thomas*, part of type (NY), *Lieutenant DuBary in 1855*, part of type (NY). I have seen no recently collected material from the Arizona side of the Colorado River and therefore include the type collections in California material. LOWER CALIFORNIA: Cajon de Santa Maria, *Brandegee in 1889* (C); Angel de la Guardia Island, *Johnston 4232* (C); San Pedro Martir Island, *Palmer 403* (C, G, NY, US), *Johnston 3147* (C); San Luis Gonzales Bay, *Johnston 3339* (C); Santa Maria, *Brandegee in 1889* (C); Santa Rosalia, *Palmer 204* (C, G, US); Cedros Island, *Rose 16161* (NY, US).

For the most part the species seems to be annual, but *Munz & Keck 9351* was certainly of longer duration and it has been pointed out by Coville (Contr. U. S. Nat. Herb. 4: 104. 1893) and Johnston (Proc. Calif. Acad. ser. 4, 12: 1121. 1924) that such may be the case. It seems to be these older plants particularly that form branches with small and closely set and more pubescent leaves than are typical. This condition is found especially in plants toward the southern part of the range, where the leaves may become very small, narrow, thin, and white-villous: *Palmer 403*, *Gilman in 1926*, *Johnston 3339*, the *Brandegee* collection at Santa Maria. The Gray Herbarium sheet of *Palmer 403* has branches with normal leaves and white-villous ones on the same sheet. So far as I can see this southern tendency is not sufficiently definite to warrant a varietal name. Greene's *O. cedrosensis* seems to be only a synonym of *O. cardiophylla* var. *typica* agreeing well with other southern material.

2b. *Oenothera cardiophylla* var. *splendens* Munz & Johnston,
Bull. Torrey Club 49: 354. 1923

Oenothera cardiophylla var. *longituba* Jepson, Man. Calif., 686. 1925.

Flowers larger, calyx-tube 20-35 mm. long; petals 13-25; style 30-60 mm. long.

Type locality, Needles, California. Material seen: Mammoth Tank (Mesquite), *S. B. & W. F. Parish 254* (G, S); Chocolate Mts., *Childs in 1920* (C); Salton Sink, near Oasis, *Jones in 1924* (P); Colorado Desert, *Pringle in 1882* (G). A local large-flowered variety confined pretty largely to the region extending from Needles south and west to Salton Sink. A collection from the Chuckwalla Mts., Riverside Co. (*Munz & Keck 4785*, C, P) is quite intermediate between *typica* and *splendens* having petals 13 mm. long, but a rather short calyx-tube, 13-15 mm. long.

3. *OENOTHERA BREVIPES* Gray, Pac. R. R. Rep. 4: 87. 1857

Annual, frequently rather coarse, usually 1- to few-stemmed from base, occasionally branched above, glabrate to spreading-villous, 10-40 cm. high, erect with nodding stem-tips; leaves largely in basal rosettes with few scattering smaller ones on lower half of stem, uppermost reduced to ovate or lanceolate bracts, lower ones (including petioles) 5-15 cm. long, glabrate to canescent to villous, frequently noticeably bicolored, with conspicuous reddish veins beneath, varying from simple with blade ca. as long as petiole and ovate to oblong-cordate and repandly denticulate, or with blade as above with few to several lance-ovate to ovate accessory leaflets on petiole, or with a pinnately compound condition with terminal leaflet not conspicuously larger than lateral ones; inflorescence a terminal somewhat pedunculate raceme, elongating in fruit to from 2 to 4 dm., glabrate to villous, usually glandular; pedicels short, 3-15 (40) mm. long, glabrous to villous; calyx-tube obconic, 3-7 mm. long, glabrous to spreading-villous without, pubescent within, at least in lower half; calyx-segments lanceolate, glabrous to villous, 6-10 mm. long, with free tips 0.5-2 mm. long; petals yellow, obovate, 7-15 mm. long; stamens somewhat shorter, the two lengths not very different, filaments glabrous, 4-8 mm. long; anthers 4-6 mm. long, with 2 rows of scattered spreading hairs; style equaling or slightly exceeding the petals, pubescent at base; stigma 1.2-2 mm. in diameter; capsules linear, 5(3)-9 cm. long, widely spreading, glabrous to villous; seeds straw-colored, obovoid, 1-1.5 mm. long, somewhat angled.

KEY TO VARIETIES

- Stem spreading-villous, usually in some part of the lower half; calyx-lobes commonly pilose as well as glandular; capsules rather thick, 2-3 mm. in diameter. South and west of Clark County, Nevada.....3a. *O. brevipes* var. *typica*.
 Stem not spreading-villous, but glabrate to finely canescent; calyx-lobes not pilose, but merely glandular-pubescent to canescent; capsules more slender, 1-1.5 mm. in diameter. Clark County, Nevada east to St. George, Utah.....3b. *O. brevipes* var. *pallidula*.

3a. *Oenothera brevipes* Gray var. *typica* n. nom.

Oenothera brevipes Gray, l. c.; Leveille, Monogr. Onoth., 143. 1905, in part; S. Wats., Proc. Am. Acad. 8: 595. 1873. *Chylismia brevipes* (Gray) Small, Bull. Torr. Club 23: 194. 1896.

Stems spreading-villous, usually in some part of the lower half; flowers bright yellow; calyx-tube with pubescent papillate structure within on each rib and just at upper edge of the pubescence; calyx-lobes commonly conspicuously pilose and finely glandular; capsules coarse, 2-3 mm. in diameter.

Type locality, Colorado River, California. Representative material: NEVADA: Rhyolite, *Heller 9677* (G), *Shockley 36* (C, S); Tonopah, *Shockley in 1907* (S); Good Springs, *Jones in 1905* (P); Mica Springs, *Jones 5036* (C, NY, P); Bunkerville, *Jones 5026* (C, P); Valley of Fire, Clark Co., *Jaeger in 1924* (P). ARIZONA: Colorado Valley, *Palmer in 1876* (C, G, Ph); Ft. Mojave, *Cooper* (G), *Jared 14* (G); Franconia, *Jones in 1903* (P); Chloride, *Jones in 1903* (P). CALIFORNIA: Pleasant Canyon, Panamints, *Hall 6994* (C, P); Darwin, *Jones in 1897* (NY, P); Funeral Mts., *Jones in*

1907 (P); Keeler, *Brandegee in 1891* (C); Kelso, *Jones in 1906* (P); Barstow, *Parish 19231* (G), *Munz 2528* (P, S); 11 miles west of Needles, *Munz & Harwood 3615* (P, W); Needles, *Jones 3818* (NY, P, W); Colorado of the West, *Bigelow on Whipple Expedition*, type collection (G, NY, Ph); Corn Springs, *Munz & Keck 4892* (P); Blythe, *Munz & Harwood 3580* (FP, P); Desert Center, *Jones in 1924* (P); Dos Palmos, *Munz 9950* (P); Canyon Springs, *Jaeger in 1926* (C); Niland, *McGregor 871* (S). The last named collection is the most southerly that I have seen.

This variety is a very variable thing. The *Jaeger* collection from the Valley of Fire has almost glabrous stems. The *Jones* collection from Kelso, the one from Darwin, *Jones 5026*, *Shockley 36* and others not above listed have unusually dense white hair on the lower part of the plant and on the buds; while *Jones 5026* has also plants with green buds. The *Brandegee* collection at Keeler suggests *O. clavaeformis* by its ascending pods, although they are 5 cm. long.

3b. *Oenothera brevipes* var. *pallidula* n. var.

Stems and leaves not spreading-villous, but glabrate to finely canescent-pubescent; flowers paler, drying reddish; calyx-tube without pubescent papillae on inner surface; calyx-lobes commonly with fine glandular pubescence only or also canescent, not pilose; pedicels commonly quite inconspicuous; capsules more slender, 1-1.5 mm. in diameter. (Caulis et folia non villosa sed glabrata aut minute canescentes-pubescentes; lobis calycis non pilosis, sed glandulosis-pubescentibus aut necnon canescentibus; capsulis tenuibus, 1-1.5 mm. latis.)

Type, Las Vegas, Nevada, *M. E. Jones on 25 April, 1905* (Pomona College Herbarium No. 38034). Ranging from Clark County, Nev. east to St. George, Utah. Material seen: UTAH: So. Utah, *Parry 73* (G, Ph); St. George, *Parry in 1874* (G), *Jones in 1880* (P), *Goodding in 1902* (W); Mokiak Pass, *Palmer 168*, in part (G). ARIZONA: Beaverdam, *Palmer 169*, in part (G). NEVADA: Moapa, *Goodding 2206* (G, W), *Kennedy 1076* (W), *Jones in 1904* (P); Indian Spring, *Tidestrom 9019* (G), *Jones in 1906* (P); Las Vegas, *Goodding 2290* (G, W), *2327* (W); Amargosa, *Heller 10977* (C, G, S); Gold Mt., *Purpus 5993* (C, W).

This variety is characterized by its ashy appearance, lack of spreading hair on the stems, simple leaves, and slender capsules. The *Goodding* collection at St. George is greenish and with a somewhat villous stem. Depauperate plants of *typica* may quite resemble *pallidula* in the lack of spreading hair, for example: Cibola, Ariz., *Grinnell in 1910* (C).

4. *OENOTHERA MULTIJUGA* S. Wats., Am. Nat. 7: 300. 1873

Annual to biennial, glabrate to closely fine-pubescent to villous, slender, one-stemmed and erect, or branching from the base and freely above, 2-8 dm. high; leaves mostly in a basal rosette or with few on the lower stems, simple or with few to many pinnae, glabrous to whitish pubescent, with conspicuous reddish veins beneath; leaves of inflorescence reduced to

minute linear or ovate bracts; inflorescence of several naked racemes in a loose open panicle 10–60 cm. long, and 5–30 cm. wide; pedicels capillary, 1–2.5 cm. long; calyx-tube funnelform, frequently pubescent within; calyx-lobes lance-ovate, often fine-glandular; petals rounded, obovate, yellow, often drying purplish; longer stamens about two-thirds as long as petals, shorter one-third; anthers linear, yellow, 1–3 mm. long, glabrous or hairy; style almost equaling petals, generally pubescent at base; stigma 0.5–1.5 mm. across; capsule linear, slender, 1–1.5 mm. in diameter and 15–35 mm. long; seeds numerous, light brown, obovoid, ca. 1 mm. long.

KEY TO VARIETIES

Flowers large, petals 7–9 mm. long, calyx-segments 6–7 mm.; anthers 2–3 mm. long, evidently hairy. Clark Co., Nevada through N. Ariz. into S. Utah.

4a. *O. multijuga* var. *typica*.

Flowers smaller, all parts less than above; anthers essentially glabrous.

Petals 3–5 mm. long, calyx-segments 3–4 mm.; leaves entirely at base of plant. Range, S.W. Utah to Inyo Co., Calif. 4b. *O. multijuga* var. *parviflora*.

Petals 1.5–2 mm. long; calyx-segments 1–1.5 mm.; leaves usually on lower third of stems, not merely at base. Range, E. Utah south into N.E. Ariz.

4c. *O. multijuga* var. *orientalis*.

4a. *Oenothera multijuga* S. Wats. var. *typica* n. nom.

Oenothera multijuga S. Wats., l. c.; Proc. Am. Acad. 8: 595. 1873.
Chylismia multijuga (S. Wats.) Small, Bull. Torr. Club 23: 193. 1896.
O. brevipes var. *multijuga* (S. Wats.) Jepson, Man. Calif., 687. 1925, in part. *O. brevipes* of Leveille, Monogr. Onoth., 446. 1913, in part.
Chylismia venosa A. Nels. & Kennedy, Muhlenbergia 3: 140. 1908. *C. hirta* A. Nels., Bot. Gaz. 47: 428. 1909.

Leaves almost entirely basal, 10–25 cm. long, generally much pinnate with 6–20 pairs of major lateral pinnae, with smaller pinnae frequently alternating, terminal pinna conspicuously larger or not; leaf-margins usually irregularly saliently dentate; petioles 2–6 cm. long; leaves glabrous to villous; flowers large; calyx-segments 6–7 mm. long, with or without free tips; petals broad, 7–9 mm. long; anthers 2–3 mm. long, with spreading hairs.

Type locality, Kanab, southern Utah. Material seen: UTAH: Kanab, Mrs. Thompson, type (G); Red Hill, north of St. George, E. M. Hall 522 (US); La Verken, Jones 5188 (C, NY, P, W); Zion Canyon, Jones in 1923 (P). ARIZONA: Beaver Dam, Palmer 169, in part (G, NY); Diamond Creek Canyon, Wilson in 1893 (P); Pierces Ferry, Jones in 1894 (P, US); without locality, Palmer in 1869 (US). NEVADA: Las Vegas, Goodding 2275 (G, W); Tuly's Ranch, Las Vegas, Goodding 2348, type coll. *hirta* (C, G, W); Muddy Valley, Lincoln Co., Kennedy & Goodding 64, type coll. *venosa* (C, NY, W); Mesquite Well, Goodding 2268 (W); Moapa, Kennedy 1818 (S, US); St. Thomas Canyon, Jones 5069 n (P, US); Eldorado Canyon, Jones in 1907 (P).

The species *multijuga* is a most perplexing affair, not only because of its several forms, but also because of the fragmentary condition of the

type and because of the intergrades between this and other species. As I understand the species, var. *typica* is the large flowered form and varies widely in the amount of pinnation in the leaves. The type consists of only a single much compounded leaf and a small flowering branch. It has always been described as having no large terminal leaflet, but I believe this due to the breaking off of the terminal member. Furthermore, there are no very mature capsules on the type; the one young one is, however, 20 mm. long on a pedicel ca. 15 mm. long; flowers on type with petals 9 mm. long. I am satisfied that this specimen represents the large-flowered group with long capsules, basal much compounded leaves, and open branching inflorescence, and includes *Chylismia hirta* and *C. venosa*, pubescence alone not being sufficient in this variable group to merit naming.

4b. *Oenothera multijuga* var. *parviflora* (S. Wats.) n. comb.

Oenothera brevipes var. *parviflora* S. Wats. ex Parry, Am. Nat. 9: 271. 1875. *Chylismia parviflora* (S. Wats.) Rydb., Fl. Rocky Mts., 603. 1064. 1917. *O. brevipes* var. *multijuga* (S. Wats.) Jepson, Man. Calif., 687. 1925 for Calif. material.

With general aspect of *typica*, but with fewer leaflets, ca. 5-8 pairs of major lateral pinnae and a larger ovate terminal one, leaves usually quite villous; flowers smaller, calyx-segments 3-4 mm. long, without conspicuous free tips; petals narrower and 3-5 mm. long; anthers not evidently hairy.

Type locality, Valley of Virgen, near St. George, Utah. Material seen: UTAH: Without locality, *Ward 249* (Ph); S. Utah, *LeRoy 168* (NY); Valley of Virgen, near St. George, *Parry 74*, type coll. (G, Ph); Mokiak Pass, *Palmer 168*, in part (G, NY); Black Rock, *Watson 414* (G); Black River, *Jones in 1881* (P); Wa Wa, *Jones in 1906* (P). ARIZONA: Grand Canyon, *Gray in 1885* (G). NEVADA: Valley of Virgen, *Purpus 6174* (C); Mesquite Well, *Goodding 2245* (G); Candelaria, *Shockley in 1881* (G), *212* (G), *408* (G); Soda Springs Canyon, *Shockley 563* (G); Meadow Valley Wash, *Jones in 1904* (P); Indian Spring, *Jones in 1906* (P); Amargosa Desert, *Jones in 1907* (P); near Logan, *Heller 10447a* (G, S); Good Springs, *Jones in 1905* (P). CALIFORNIA: Furnace Creek, *Parish 10043* (S), *10045* (C); Darwin, *Jones in 1897* (P); Inyo, *T. S. Brandegee in 1891* (C); Argus Mts., *Jones in 1897* (P); Kelso, *Jones in 1906* (P).

Originally placed with *O. brevipes* this variety belongs rather to *O. multijuga* with its long pedicels, pinnate leaves, numerous branches and slender capsules. It is not altogether uniform; *Heller 10447a* and *Purpus 6174* have short capsules, 10-25 mm. long. *Watson 414* and the *Jones* collection at Black River, Utah are atypical in the small number of pinnae and in being less hairy than usual; in fact they suggest *O. scapoidea* but have longer capsules and branch rather freely in the upper part of the plant.

4c. *Oenothera multijuga* var. *orientalis* n. var.

Leaves not only basal, but usually some part way up the stems, frequently with few if any lateral pinnules, margin shallowly dentate; flowers small, calyx-segments 1-1.5 mm. long; petals 1.5-2 mm.; capsules 15-20 mm. long. (Flores minuti; lobis calycis 1-1.5 mm. longis; petalis 1.5-2 mm. longis.)

Type from Moab, Utah, *M. E. Jones* on 7 June, 1913 (Pomona College Herbarium No. 38608). Material seen: COLORADO: Sand Bar of Rio Mancos, *Brandegee* 1049 (C). UTAH: Logan, *C. P. Smith* 1988 (W); Thompsons Springs, *Jones* in 1913 (P); Green River, *Jones* in 1914 (P); Grand River Crossing opposite Moab, *Rydberg & Garrett* 8361 (NY, US, W); Moab, *Eastwood* in 1892 (C, G). ARIZONA: Lee's Ferry, *Jones* in 1890 (C, P, US); Grand Canyon, *A. E. Hitchcock* 83 (US); Cameron, *Hanson* 1118 (NY).

The short capsules, small flowers and lack of pinnules show a close affinity to *O. scapoidea*, but the hairiness of the plant, the slender capsules, and the dark color of the dry flowers suggest *multijuga*. Collections from Grand Junction, Colorado, *Eastwood* in 1892 (C, G, US) and *Jones* in 1883 (P) are quite intermediate between var. *orientalis* and *O. scapoidea* in having leaves scarcely pinnatifid, pubescence short and fine, capsules like those of *scapoidea*; but the purplish flower color when dry and lack of hair on anthers are like *orientalis*. The collection from Paradox, Colo., *Walker* 200, type of *Chylismia Walkeri* A. Nels. (G, S, W), is another intergrade with *scapoidea*, with the pubescence and flowers of *orientalis* and with the short capsules of *scapoidea*. It is almost exactly matched by material from Echo Cliffs, N. Ariz., *Jaeger* in 1927 (P). A form in the Grand Canyon of the Colorado, *Toumey* 139 (S, US), *Wootton* in 1892 (NY) has much pinnate leaves, a low habit (10-20 cm.), short capsules (10-12 mm.) and small flowers (petals 2-3 mm.) and suggests *parviflora*, *orientalis*, and *scapoidea*. *Jones*, in 1890 (P), collected at the Buckskin Mts., Ariz., a form which is quite smooth, branching and with scattered leaves and with flowers of *orientalis* and capsules of *Parryi*. It is evident then, that there are considerable variation and intergradation.

5. *OENOTHERA SCAPOIDEA* Nutt. ex T. & G., Fl. N. Am. 1: 506. 1838-1840

Annual, simple or branching from base, erect or spreading, glabrous to finely pubescent or glandular-puberulent, 10-45 cm. high; stems rather slender, scarcely if at all branched; leaves mostly at base of plant or basal and on lower part of stems only, prevailing simple, occasionally with few pinnules along petioles, ovate to oblong-ovate, cuneate or subcordate at base, obtuse, subentire or coarsely and irregularly dentate, not conspicuously bicolored, 1-4 cm. long, 0.5-3 cm. wide; petioles 2-7 cm. long, often somewhat winged; upper leaves reduced to sessile ovate to lance-ovate bracts; inflorescence mostly racemose, occasionally branched and forming slender panicles, constituting the upper half to three-fourths of the plant; pedicels spreading or ascending, capillary, 0.5-1.5 cm. long;

calyx-tube obconic to short-funnelform, 1.5–3 mm. long, glabrate to finely pubescent within; calyx-segments lance-oblong to ovate, 2–6 mm. long, without free tips; petals rounded, orbicular to obovate, 2–10 mm. long, yellow, frequently with reddish dots at the base; longer stamens ca. equal to petals, shorter ones about two-thirds as long; anthers oblong-linear, 1–3.5 mm. long, with scattered white hairs; style equal to or longer than petals, sparsely pubescent near base; stigma 0.5–1 mm. wide; capsules quite erect, clavate, slightly curved, 10–25 mm. long, 2–2.5 wide; seeds brownish obovoid, 1.5–2 mm. long.

KEY TO VARIETIES

Flowers small; petals 2–4 mm. long; calyx-segments 2–3 mm. long.

Stems and inflorescence glabrous.....5a. *O. scapoidea* var. *typica*.

Stems and inflorescence glandular-puberulent.....5b. *O. scapoidea* var. *seorsa*.

Flowers larger; petals 8–10 mm. long; calyx-segments 6 mm. long.

5c. *O. scapoidea* var. *Eastwoodae*.

5a. *Oenothera scapoidea* Nutt. var. *typica* n. nom.

Oenothera scapoidea Nutt., l. c.; Wats., Proc. Am. Acad. 8: 595. 1873.
Chylismia scapoidea (Nutt.) Small, Bull. Torr. Club 23: 193. 1896.
O. brevipes var. *scapoidea* of Leveille, Monogr. Onoth. 146. 1905, in part.

Stem and inflorescence glabrous; petals 3–4 mm. long; calyx-segments 2–3 mm.; anthers ca. 1.5 mm. long; style 4–8 mm. long; seeds ca. 1.5 mm.

Type locality, "clay hills in the Rocky Mountains, Nuttall." Material seen: ROCKY MTS., Nuttall (G, NY, Ph). WYOMING: Ft. Steele, Nelson 4813 (W), Tweedy 4436 (NY); Worland, Buffum in 1909 (W); Green River, Jones in 1896 (P), Rydberg in 1895 (NY), Parry 39 (G), 112 (G, Ph), Nelson 3025 (G, NY, P, W); Alcova, Goodding 148 (G, C, NY, P, S, W); Leckie, Merrill & Wilcox 748 (G, NY, W); Granger, Nelson 4702 (C, W), 7227 (G, NY, P, W). UTAH: Green River, Jones in 1890 (P); Detroit, Jones in 1891 (P); Marysville, Jones 5388g (C, NY, P); Juab, Goodding 1059 (G, P); Vermilion, Jones in 1901 (P); Chepeta Well, Jones in 1908 (P); Randelet, Jones in 1908 (P); Wasatch Mts., Watson 414 (NY). NEVADA: Deeth, Heller 10566 (G, NY, S); Cobre, Jones in 1906 (P). Intergrades between *scapoidea* and *multijuga* have already been discussed. *O. scapoidea typica* and var. *seorsa* are not clearly marked, but there does seem to be enough difference in the extreme forms to warrant maintaining Nelson's variety *seorsa*.

5b. *Oenothera scapoidea* var. *seorsa* (A. Nels.) n. comb.

Chylismia scapoidea var. *seorsa* A. Nels., Bot. Gaz. 54: 140. 1912.

Stems and inflorescence glandular-puberulent; flowers often smaller than in *typica*; calyx-segments ca. 2 mm. long; petals 2–3 (4) mm.; style 4–6 mm. long.

Type locality, Evanston, Wyo. Material to be referred here: WYOMING: Evanston, Nelson 4125, type (W); Solon, Williams in 1897 (S, W); Ft.

Bridger, *Porter in 1873* (G, NY, Ph); Dayton, *Tweedy 2608* (NY). COLORADO: Cimarron, *Baker 72* (G, NY, P, S, W, WS); Canon City Park, *Brandege 927* (C); Grand Junction, *Eastwood 5214* (S); Hotchkiss, *Cowen in 1892* (G, W, WS); Montrose, *Payson 34* (W); Naturita, *Payson 308* (G, W); Mesa Co., *Long in 1893* (G); Palisade, *Crandall 18* (NY). UTAH: Milford, *Jones 1778* (C, NY, P, W); Thompsons Springs, *Jones in 1913* (P); Sunnyside, *Jones in 1905* (P); Emery, *Jones 5458b* (P); Toole Valley, *Garrett 2759* (NY); Desert Station, *Rydberg & Garrett 8318* (NY). IDAHO: Challis Creek, Custer Co., *Macbride & Payson 3341* (C, G, NY, P, S, W); Glenn's Ferry, *Jones in 1911* (P); King Hill, Elmore Co., *Nelson & Macbride 1145* (G, S, W); Salmon, *E. B. & L. B. Payson 1793* (G, NY, W). OREGON: without locality, *Geyer 94* (G); Malheur, Baker Co., *Cusick 1086* (G). At best this variety is an ill-defined one, its two characters of small flowers and glandular pubescence not always occurring together; for example most Colorado material has the glandular pubescence, but flowers of normal size.

5c. *Oenothera scapoidea* var. *Eastwoodae* n. var.

Inflorescence glabrate to glandular-puberulent; flowers large; calyx-tube 3 mm. long; calyx-segments ca. 6 mm. long; petals 8–10 mm.; anthers ca. 3.5 mm. long; style 12–14 mm.; seeds 2 mm. (Flores magni; tubo calycis 6 mm. longo; lobis calycis 6 mm. longis; petalis 8–10 mm.; antheris 3.5 mm. longis; stylo 12–14 mm.; seminibus 2 mm. longis.)

Type, Grand Junction, Colorado, *A. Eastwood, May, 1892* (at Gray Herbarium, photograph at Pomona College; isotype at University of California. Other material seen: COLORADO: Westwater, *Jones in 1891* (P); Grand Junction, *Stokes in 1900* (S). UTAH: Green River, *Jones 5482d* (P). A well-marked variety with its large flowers. The open habit, glabrous condition, entire basal leaves, etc. are indicative of close affinity to *O. scapoidea* var. *typica*.

6. *OENOTHERA CLAVAEFORMIS* Torr. & Fremont, *Frem. Rep.*, 314. 1845

Annual, simple or usually with few unbranched stems from the base, 10–30(50) cm. tall, glabrate to pubescent to villous, sometimes glandular above, often reddish; leaves mostly in basal rosette, simple and irregularly shallowly dentate with ovate blades 2–5 cm. long and 1–3 cm. wide and with petioles of about same length, or with few small pinnae along petiole, or with terminal portion lobed and with many supplementary pinnae; leaves glabrous to canescent-pubescent to villous, with cauline leaves reduced to ovate bracts 5–10 mm. long, those of inflorescence quite minute; inflorescence racemose, somewhat peduncled, flowers during anthesis quite crowded, later more scattered; pedicels 8–25 mm. long, slender; calyx-tube obconic, glabrous to strigillose to villous without, pubescent within, ca. 5 mm. long, commonly brownish or colored orange within; calyx-lobes lance-ovate, 4–5 mm. long, glabrous to strigillose to villose, usually with minute free tips; petals orbicular-obovate, 4–7 mm. long, white or yellow, often drying reddish; stamens almost equal, filaments ca. two-thirds the

length of the petals, 4-5 mm. long, with white spreading hairs; pistil equaling or exceeding petals, pubescent at base, 11-15 mm. long; stigma ca. 1 mm. across; capsule clavate, commonly almost 2 mm. thick, 12-20 (25) mm. long, generally curved and ascending; seeds light brown, obovoid, somewhat angled, 1.2 mm. long.

The heavier stems, more dense flower clusters and less differentiated stamens, as well as rather distinct range argue for the specific rank of the plants here included which can be separated into four varieties as follows:

KEY TO VARIETIES

Flowers whitish, often brown at throat; petals drying purplish.

Calyx-lobes, ovaries and upper parts of stems glabrous; leaves usually not much pinnatifid. 6b. *O. clavaeformis* var. *typica*.

Calyx-lobes, ovaries and upper parts strigillose-pubescent; leaves usually much pinnatifid. 6c. *O. clavaeformis* var. *aurantiaca*.

Flowers yellow.

Leaves scarcely if at all pinnatifid; stems glabrate to canescent-puberulent. Inyo County, Calif. northward into eastern Oregon. . . 6a. *O. clavaeformis* var. *cruciformis*.

Leaves much pinnatifid; stems spreading-villous. Imperial Co., California.

6d. *O. clavaeformis* var. *Peirsonii*.

6a. *Oenothera clavaeformis* var. *cruciformis* (Kell.) n. comb.

Oenothera cruciformis Kell., Proc. Calif. Acad. 2: 227, f. 71. 1863. *Chylismia cruciformis* (Kell.) Howell, Fl. N. W. Amer., 233. 1898. *Chylismia scapoidea* var. *cruciformis* (Kell.) Small, Bull. Torr. Club 23: 193. 1896. *O. scapoidea* var. *purpurascens* S. Wats., Proc. Am. Acad. 8: 595. 1873, as to plants cited. *Chylismia lancifolia* Heller, Muhlenbergia 2: 226. 1906.

Stems commonly closely and finely canescent-puberulent; flowers clear yellow or with reddish spots; leaves scarcely or not at all pinnatifid.

Type locality, "beds of silicious deposit on the top of Steamboat Springs, Nevada." Ranging from eastern Oregon along the east base of the Sierra Nevada to Washoe Co., Nevada and Inyo Co., California. Representative material: OREGON: E. Ore., *Cusick* 1944 (C, G, WS); Mathew Butte, *Leiberg* 2042 (C, G, NY, P); base of Steins Mts., *Howell* 401 (G, NY, Ph). NEVADA: near Reno, *Heller* 9708 (G, NY, Ph, S), *Jones* in 1897 (P); Truckee Pass, *Kennedy* 2059a (S); Truckee Valley, *Bailey* 415 (G); Glendale, *Kennedy* 1574 (C, NY, US); Steamboat Springs, *Sonne* in 1887 (C); Pyramid Lake, *Kennedy* 1034 (C, NY); Unionville Valley, *Watson* 415 (G); Carson City, *Anderson* 70 (G). CALIFORNIA: Honey Lake Valley, Lassen Co., *Davy* 3399 (C); Amedee, *Jones* in 1897 (P); Volcanic Tableland, Mono Co., *Peirson* 6036 (FP, P); Rock Creek, *Ferris* 1399 (S); Mono Lake, *Brewer* 1845 (C, G); McGee's Meadows near Bishop, *K. Brandegee* (C); Bishop Creek, *Hall & Chandler* 7265 (C, P, W); White Mts. E of Laws, *Heller* 8231, type collection of *lancifolia* (C, G, NY, Ph, S); Crooked Creek, Owens Valley, *Peirson* 790 (FP); Canon of the Truckee, *K. Brandegee* in 1913 (C, G, W).

In some ways it would seem best to use the varietal name *purpurascens* S. Wats. instead of *cruciformis* for this group, since that name seems to be the oldest varietal name used. But Watson in his description, Proc. Am. Acad. 1. c., gives the flower color as usually white, rarely yellow, and seems to have in mind the characters of what I am calling var. *typica*. Yet he gives, as the range for his variety, eastern Oregon south to Mono Lake, distinctly the range of the yellow-flowered *cruciformis* and the collections he cites are yellow-flowered. Because of the confusion which would result from using his name *purpurascens* for a yellow-flowered plant, I am taking a name about which there can be no doubt. It is sometimes difficult to tell flower color in dried plants, but these are plants that Watson had seen growing.

Cruciformis intergrades with *typica* in the region of Randsburg, Kern Co., Calif., as evidenced by both yellow and white-flowered plants having been collected there by K. Brandegee in 1913 (C).

6b. *Oenothera clavaeformis* Torr. & Frem. var **typica** n. nom.

O. clavaeformis Torr. & Frem., Rep., 314. 1845. *Chylismia clavaeformis* (Torr. & Frem.) Heller, Muhlenbergia 2: 105. 1906. *Oenothera scapoidea* var. *clavaeformis* (Torr. & Frem.) S. Wats., Bot. King Exped., 109. 1871, in part. Probably *O. scapoidea* var. *tortilis* Jepson, Man. Calif., 687. 1925.

Stems glabrate to finely pubescent; flowers whitish, often with reddish or brownish, more or less cruciform patch at upper edge of calyx-tube, petals often drying purplish; leaves from simple to somewhat pinnatifid; buds and inflorescence glabrate, sometimes finely glandular.

Type locality, on Mohave Desert between Cajon Pass, California and Las Vegas, Nevada. Range, western half of Mohave Desert through Kern and Inyo Counties to central Nevada; apparently usually found above 2500 ft. altitude. Representative material: NEVADA: Candelaria, Shockley 411 (G); Palmetto Range, Purpus 5892 (C, P); Austin, Kennedy 4559 (G); between Pyramid and Winnemucca Lakes, Kennedy 2040 (Ph, S, W); Palisade, Jones 3869 (NY, P), Heller 9943 (C, S). CALIFORNIA: Fremont's Pac. R. R. Exp., 1843-'44, "probably collected in California," type (NY); Panamint Valley, Coville & Funston 664 (G, NY, S); Argus Peak, Hall & Chandler 6893 (C, P, W); Garlic Springs, Munz & Keck 7891 (P); Keeler, T. S. Brandegee in 1891 (C); Daggett, Munz & Harwood 3653 (P); Barstow, Parish 9731 (S), 9732 (S), Munz 2566 (P); Victorville, Johnston in 1920 (P); Acton, Elmer 4173 (NY); Rock Creek, Peirson 6664 (FP); Mojave, Munz 10063 (P); Randsburg, Heller 7691 (C, G, S). Some collections, such as Purpus 5892 are slightly strigillose and intergrade with the var. *aurantiaca*. Twisted pods, the character used by Jepson for his var. *tortilis*, are not uncommon in *clavaeformis*.

6c. *Oenothera claviformis* var. *aurantiaca* (S. Wats.) n. comb.

O. scapoidea var. *aurantiaca* S. Wats., Proc. Am. Acad. 8: 595. 1873, in part. *Chylismia scapoidea* var. *aurantiaca* (combination incorrectly ascribed to Wats. by) Davidson & Moxley, Fl. S. Calif., 254. 1923.

Stems glabrate to finely pubescent; flowers like those of *typica*, mostly whitish and often drying purplish, but sometimes a pale yellow when fresh; calyx-lobes and inflorescence finely strigillose-pubescent; leaves tending to be much pinnate (especially in plants from Needles southward).

The characters given in Watson's description leave no doubt as to what his var. *aurantiaca* is, but in selecting a type, I have decided not to use the first specimens cited ("*Coulter 180, in part*") since that collection seems to include var. *aurantiaca*, var. *Peirsonii*, and possibly some material of *O. brevipes*. In arbitrarily selecting a type therefore, it seems wise to take a collection made at Ft. Mojave, Arizona by *Cooper in 1861* (C, G), one of the collections cited by Watson and about which there can be no uncertainty. The variety ranges at low altitudes, apparently mostly below 2000 ft., in the desert region from Death Valley and the Colorado Desert to St. George, Utah. Representative material: UTAH: S. Utah, *Parry in 1874* (G); Central Union Pacific R. R., *T. S. Brandegee in 1882* (C). NEVADA: Tonopah, *Shockley 88* (S); Currant, *Bentley in 1916* (P, S, W); Calientes, *Goodding 940* (W); Amargosa Desert, *Jones in 1907* (P). ARIZONA: Santa Cruz River, *W. F. Parish 57* (NY); Benson, *Brandegee in 1882* (S); Tucson, *Toumey in 1892* (C, S); Yuma, *Thomas in 1855* (NY), *Goldman 1079* (US); Congress Junction, *Jones in 1903* (P); Wickenburg, *Jones in 1903* (P); Beaverdam Wash, *Goodding 2142* (G, W). CALIFORNIA: Ludlow, *Jones in 1926* (P), *Munz & Harwood 3409* (P); Ash Hill, *Hall 6089* (C, S); Bagdad, *Wilcox in 1905* (US); Funeral Mts., *Jones in 1907* (P), *Coville & Funston 447* (G, NY, S, US, Ph); Furnace Creek, *Parish 10033* (S), *Coville & Funston 571* (G, NY, S); Silver Lake, *Munz & Keck 7897* (P); Needles, *Jones 3793* (C, NY, P, W); east of Riverside Mts., *MacDougal 45* (NY); Painted Canyon, *McGregor 729* (S); Corn Springs, *Munz & Keck 4913* (P); Mecca, *Hall 5835* (C); Thousand Palms, *Munz 10822* (P); Palm Springs, *Eastwood 2983* (G, NY, P); Yaqui Well, *Jones in 1926* (P); Ogilby Station, *Mallory in 1920*; Old Beach, *Abrams 3199* (G, NY, Ph, S). SONORA: Nocha Buena Mesa, *MacDougal in 1904* (NY).

It is not surprising that in a group covering so wide a territory there should be considerable variation. Some of the peculiar forms are as follows: Amargosa Desert, *Jones in 1907* and Cave Spring, *Jones in 1924* (P) have peculiar dark red flowers and the first named has an unusual, tall branching stem. *Rose 11877* from Tucson, Ariz. (US), *Wilcox in 1905*, from Benson, Ariz. (US), and *Wootton in 1912* from Santa Rita Range Reserve, Ariz. (US) are all glandular as well as pubescent. A considerable number of plants are suggestive of *O. brevipes* and may be hybrids with that species: Mecca, *Hall 5833* (C) has small flowers and long pedicels of

aurantiaca but the less congested inflorescence and longer capsules of *brevipes*. *Munz* 9973 from Dos Palms (P) has pale yellow flowers, not crowded, with petals ca. 8 mm. long, capsules 30 mm., pedicels 10–20 mm., and non-villous stems, thus suggesting both; it is well matched by *Munz & Keck* 7937 from Garlic Springs (P); *Munz & Harwood* 3617 from west of Needles (P, S); *Parish* 10015 from Furnace Creek (S); *Hall* 6115 from Hectors (C). Another series has flowers yellow, petals 6 mm. long; leaves villous, stems slightly so, pedicels short, 3–5 mm., and capsules 25–40 mm. long; this is exemplified by *Hall* 5974, from Hodges Mts., Colorado Desert (C).

6d. *Oenothera clavaeformis* var. *Peirsonii* n. var.

Stems spreading-villous; leaves often much divided; flowers yellowish. (Caules villosi; foliis saepe multum divisis; floribus luteis).

Type, from Imperial County, California, 28 miles south of Coachella, 12 April, 1922, *F. W. Peirson* 4512 (Pomona College Herbarium No. 138409. Isotype in Frank Peirson Herbarium). Ranging through the western part of the Colorado Desert of California from Borrego Valley south into northern Lower California. Material seen: CALIFORNIA: without locality, *Coulter* 180, in part (G, NY); Coyote Canyon, *Hall* 2815 (C, NY); east end of Santa Rosa Mts., *Peirson* 7181 (FP); Borregos Springs, *K. Brandegee* in 1899 (C, G, NY, W), *Jones* in 1906 (P); Cholla Ranch, *Jones* in 1906 (P); San Felipe Wash, *McGregor* 750 (S); Vallecitos, *Thurber* 643 (G); Yaqui Wells, *Eastwood* 2655 (G, NY); Coyote Wells, *Feudge* 1359 (P), *Jaeger* in 1925 (P), *Spencer* 243 (P), *McGregor* 838 (S); Mountain Springs, *Schoenfeldt* 3103 (S), 3194 (S), *Parish* 9091 (S), *Peirson* 2892 (FP); Signal Mt., *Abrams* 3168 (NY, S), *T. S. Brandegee* in 1901 (C). LOWER CALIFORNIA: San Fernando, *T. S. Brandegee* in 1889 (C); Calamuguet, *T. S. Brandegee* in 1889 (C).

Intergrades with var. *aurantiaca* are from Yaqui Well, *Jones* in 1926 (P) and from 25 miles east of Holtville, *Munz* 7813 (P), with rather smooth stems but yellow flowers.

7. *OENOTHERA HETEROCHROMA* S. Wats., Proc. Am. Acad. 17: 373. 1882

Chylismia heterochroma (S. Wats.) Small, Bull. Torr. Club 23: 193. 1896. *Oenothera brevipes* race *Parryi* (S. Wats.) Leveille, Monogr. Onoth., 146. 1905, in part.

Apparently annual, simple or branched at the base, branching above, glandular-pubescent throughout, 25–50 cm. high; leaves in lower portion only, but not usually in basal rosette, ovate, rounded or somewhat cordate at base, acute to obtuse, irregularly serrate, with fairly conspicuous veins beneath, villous, 2–6 cm. long, 1.5–4 wide, on petioles 1–5 cm. long, upper leaves reduced, subsessile; inflorescence open, paniculate, with slender racemose branches; pedicels capillary, 2–5 mm. long; calyx-tube funnel-form, 2.5 mm. long, glabrous within; calyx-lobes lanceolate, 2.5 mm. long; petals obovate, 3–5 mm. long, purplish; longer stamens about half the

length of the petals, shorter ones about one-third; anthers oblong, less than one and one-half times as long as wide, ca. 0.6 mm. long; style almost equal to petals, pubescent near base; stigma ca. 1 mm. across; capsule 8-13 mm. long, ca. 2 mm. thick, clavate; seeds brown, obovoid, 1 mm. long.

Type locality, Candelaria, Nevada. Material seen: NEVADA: Candelaria, *Shockley in 1881*, No. 19, type (G), 562 (G, NY, P, S); Soda Springs Canyon, *Shockley 561* (C, G, NY, S); Silver Peak, *Purpus 6419* (W); Tonopah, *Shockley 120* (P, S); Panaca, *Jones in 1912* (P); Fallon, *Mrs. Ross* (C); Goldfield, *Solms in 1908* (G). CALIFORNIA: Crooked Creek, Owens Valley, *Peirson 795* (FP). There is a slight tendency for some of the more westerly collections, such as those from Tonopah and Fallon, to have unusually large flowers, with petals 4-5 mm. long, while the California collection and the more easterly Nevada ones have petals from 3-4 mm. long.

8. *OENOTHERA PARRYI* S. Wats., Am. Nat. 9: 19 & 270. 1875

Chylismia Parryi (S. Wats.) Small, Bull. Torr. Club 23: 193. 1896. *Oenothera scapoidea* var. *Parryi* (S. Wats.) Jones, Proc. Calif. Acad., ser. 2, 5: 682. 1895. *O. brevipes* race *Parryi* (S. Wats.) Leveille, Monogr. Onoth., 146. 1905, in part. *O. tenuissima* M. E. Jones, Proc. Calif. Acad., ser. 2, 5: 683. 1895. *Chylismia tenuissima* (M. E. Jones) Rydb., Bull. Torr. Club 40: 66. 1913.

Annual, 10-80 cm. high, erect, simple at very base and branching profusely part way up, or branching freely throughout, whole lower portion villous with spreading hair; leaves rather crowded on lower parts of branches, or well distributed, lower ones lanceolate to oblong-ovate, bi-colored, glabrate above, villous beneath, 1-2.5 cm. long, 0.5-1.5 wide, cuneate to subcordate at base, acute to obtuse, subentire to "subsiniately toothed"; petioles slender, 1-3.5 cm. long; uppermost leaves reduced to minute ovate bracts; inflorescence paniculate, spreading, with numerous fine, glandular-pubescent branches; pedicels capillary, recurved, 0.5-1.6 cm. long; calyx-tube 1-2 mm. long, funnellform, glabrous; calyx-lobes 2-4 mm. long, lance-oblong, without free tips, glandular-puberulent with scattered longer hairs; petals suborbicular, 3-7 mm. long, deep yellow or orange, occasionally spotted with red inside; longer stamens ca. two-thirds the length of the petals; shorter ones ca. half; anthers linear, 1-1.5 mm. long, glabrous; style almost equal to petals, glabrous; stigma ca. 0.5 mm. broad; capsules clavate, 3-9 mm. long, 1.5 mm. wide; seeds light brown, obovoid, 0.7-1.0 mm. long.

Type locality, bare clay hills near St. George, Utah. Material seen: UTAH: St. George, *Parry 72*, type collection (G, Ph, S), *Palmer 167* (G, NY, US); southern Utah, *LeRoy 167* (NY); Mokiak Pass, *Jones in 1927* (P); Rockville, *Jones 6083*, in 1894, type coll. of *tenuissima* (C, NY, P, W), in 1925 (P). Very few collections have been made of this interesting species, but from the material at hand it is evident that *tenuissima* is merely a small-flowered and short-capsuled form of *Parryi*. The 1927 collection

made by *Jones* shows every degree of intermediacy. The type of *Parryi* was quite immature, that of *tenuissima* was collected in September and was very mature with basal leaves gone. The 1925 collection of *Jones* at Rockville was made earlier in the season and shows basal leaves. *Palmer 167* has perhaps the largest flowers of any specimen seen and is at the other end of the series from *tenuissima*.

DOUBTFUL SPECIES

OENOTHERA (CHYLISMIA) DIVARICATA Greene, Bull. Torr. Club

10: 125. 1883

The next to the last line of the description is apparently omitted, so that it is not perfectly plain as to what the author wished to say about the seeds, which are said to be narrowly winged. Aside from this character the description fits *O. brevipes* very well. Immature seeds of that species do appear somewhat winged.

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THE DEVELOPMENT OF THE PERITHECIUM AND SPERMAGONIUM OF *SPORORMIA LEPORINA* NIESSL

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This paper is based on a study of the development of the perithecium and spermagonium of *Sporormia leporina*, a pyrenomycete belonging to the Sordariaceae. Some striking peculiarities are shown in the development of the fruiting structures.

METHODS

Cultures of the fungus were made on plates of plain, unneutralized potato agar, and since this medium is unfavorable for bacterial growth little difficulty was experienced in avoiding contamination.

When fruiting structures appeared in the cultures blocks of agar bearing the fungus were removed and placed in fixing fluid. The fixers used were the chromo-acetic—osmic acid and the corrosive sublimate-formalin—acetic acid solutions. In general the former gave the best fixation. The material was then imbedded in paraffin and sections were cut 5 to 10 microns in thickness. Sections cut parallel to the surface of the plate were most satisfactory for studying the early stages. Such sections showed the relation of the young fruiting structures to the vegetative mycelium much better than did sections cut at right angles to the surface.

Delafield's haematoxylin was a satisfactory stain for the gross morphology of the perithecium. Heidenhain's iron-alum haematoxylin with a counterstain of erythrosin was the best for detail.

DEVELOPMENT OF THE FRUITING STRUCTURES

Spermatogonia appeared when the cultures were about two weeks old. Perithecia appeared a few days later, and at first the two were indistinguishable to the unaided eye.

The perithecia originate as single swollen cells which appear scattered throughout the vegetative mycelium (Pl. XV, fig. 1). These cells are about thirteen microns in diameter or two or three times the diameter of the vegetative cells. In all determinable cases each swollen cell contains one nucleus. Within each of these cells cross walls form at right angles to each other, dividing the cell into four parts (figs. 2, 3, 4). Other divisions occur and when the structure consists of fifteen to twenty cells it commences to enlarge. The cells of the interior are small, thin walled, with weakly staining cytoplasm and inconspicuous nuclei. The walls of the outer layer

of cells become deep brown and when examined in culture at this stage the perithecium appears as a small knot of brown hyphae (fig. 6).

When the perithecium reaches a diameter of about twenty microns some internal differentiation occurs. The interior becomes rather loosely arranged due to the pulling apart of the inner cells (fig. 7 *b*). The cells of the upper portion of the interior (fig. 7 *a*) remain more compact, are smaller, and stain more deeply than do those below. Immediately below this area of compact smaller cells are a few cells which are larger than the others (fig. 7 *e*), and in slightly older stages of the perithecium they are attached to the ends of hyphal strands (fig. 8).

Surrounding this loosely arranged inner portion but immediately within the outer enclosing layer of brown cells is a layer of discontinuous, smaller, lightly stained cells (fig. 7 *c*) which, in the mature perithecium, is seen to have increased to form the main part of the perithecial wall. As the perithecium enlarges still more a cavity forms within (Pl. XVI, fig. 15), and surrounding this is a layer four or five cells in thickness which has arisen from the former discontinuous layer. This layer now composes the inner and thicker part of the perithecial wall.

As the perithecium continues to grow the wall thickens. The central cavity soon becomes filled with hyphae which occupy a vertical position and extend downward from the upper part of the inner surface of the wall (Pl. XVI, figs. 15, 16, 18). There is no evidence of any attachment of these anywhere except at the top of the cavity, and the lower ends hang free. These hyphae are several cells in length and each cell contains one nucleus. The terminal cells of some of these hyphae are shorter and of greater diameter than the intercalary cells and are very similar to some of the large cells in the loosened interior during the earlier stages (Pl. XV, figs. 7-9).

As the perithecium grows and the vertical hyphae become more numerous the enlarged cells at their tips form a rather definite zone across the lower part of the cavity (figs. 18-20). As a result of the increase in length of the vertical hyphae this layer at their tips is pressed firmly against the bottom of the cavity and in a mature perithecium it is difficult to demonstrate that it is attached to the vertical hyphae rather than to the wall at the bottom (figs. 20, 21). When the larger perithecia are dissected with needles under a dissecting microscope it is possible to remove the mass of large cells from the cavity still attached to the lower ends of the vertical hyphae which in turn tend to break loose from the top of the cavity with frayed ends.

It is from these enlarged cells at the tips of the vertical hyphae that the ascogenous hyphae appear to arise. They elongate upward, *i.e.* in the opposite direction from that of the vertical hyphae. The first ascospores are delimited when the perithecium is about one-half mature. No true paraphyses have been observed.

Some of these enlarged cells in a half matured perithecium contain two nuclei (figs. 10, 11). Such binucleate cells were not numerous but their attachment to the vertical hyphae could be observed in a few cases (fig. 10). These nuclei are no larger than the nuclei of the cells of the vertical hyphae and they appear as deeply staining dots in the cytoplasm. Pairs of minute nuclei could be observed in the penultimate cell of the ascus hooks (fig. 5). It was difficult to demonstrate the actual attachment of the ascogenous hyphae to the enlarged cells at the tips of the vertical hyphae because they are small and thin walled and in growing upright they seem to follow the path of least resistance among the vertical hyphae with the consequence that they seldom grow perfectly upright, so it is impossible to follow their course in thin sections.

The vertical hyphae first become conspicuous when the perithecium has reached approximately one-fourth of its mature diameter, but as the perithecium grows the zone of attachment of the vertical hyphae remains confined to a rather definite area at the apex (figs. 16, 18).

The spermatogonia arise from rhizomorphs containing eight to a dozen hyphae which are intermingled with the ordinary vegetative mycelium. At places in a rhizomorph short cells are cut off followed by the formation of a knot (fig. 12). This knot continues to enlarge by cell division until a structure results which resembles a young perithecium before the formation of the cavity (fig. 13). It can be distinguished from the perithecium by its attachment to the rhizomorph. As it continues to grow, small deeply staining spermatia develop in a gelatinous mass in the interior (figs. 14, 17). The spermatia are one and one-half microns in length and more than half of the cell is occupied by the nucleus. The spermatia escape from the spermatogonium in a gelatinous mass in the same manner as in *Sordaria coprophila* (Woronin, 6).

DISCUSSION

Dangeard (1) reports a swollen, multinucleate cell as the perithecial initial of *Sporormia intermedia*. Satin (5) studied the same form but did not observe the swollen cells. According to both accounts, as the perithecium enlarges, a cavity forms within as in *S. leporina*. According to Dangeard and Satin, deeply staining and somewhat enlarged cells then appear at the bottom of the cavity and from them ascogenous hyphae and paraphyses arise. Similar enlarged cells (fig. 7 e) appear at the time of cavity formation in *S. leporina* but they differ from those described by Dangeard and Satin by appearing to be the enlarged cells which, in later stages of the perithecial development, occur at the tips of the vertical hyphae. Therefore the outstanding difference between the enlarged cells in the young perithecium of the two species is their mode of attachment, i.e. at the bottom of the cavity in *S. intermedia* and at the lower ends of the young vertical hyphae in *S. leporina*.

The nuclear condition of the enlarged cells of the very early stages of the perithecium of *S. leporina* could not be determined. The perithecial initial of *S. intermedia*, according to Dangeard, is a multinucleate cell, while there is but one nucleus in the corresponding stage of *S. leporina*, and there appears to be but one nucleus per cell even after several cell divisions have occurred (fig. 2).

The most conspicuous difference in the morphology of the perithecium of the two species is in the arrangement of the structures within the cavity as maturity approaches. Instead of the cavity being filled with asci and paraphyses arising from enlarged cells attached to the bottom of the cavity as in *S. intermedia*, the cavity of *S. leporina* is filled with downward growing vertical hyphae, and then from the region of their tips ascogenous hyphae grow upward and in the opposite direction (figs. 19-21).

Hartig (4) figures hyphae growing downward into the cavity of the perithecium of *Rosellinia quercina*. In this case, however, he figures asci arising from cells attached at the base and around the sides of the cavity, and they are in no way connected with the hyphae growing downward from the top.

Griffiths (2, 3) separates the species of *Sporormia* into two groups on the presence or absence of paraphyses, and *S. leporina* is placed in the group with these present. On superficial examination the vertical hyphae closely resemble paraphyses, but because of their origin and attachment they cannot be so considered.

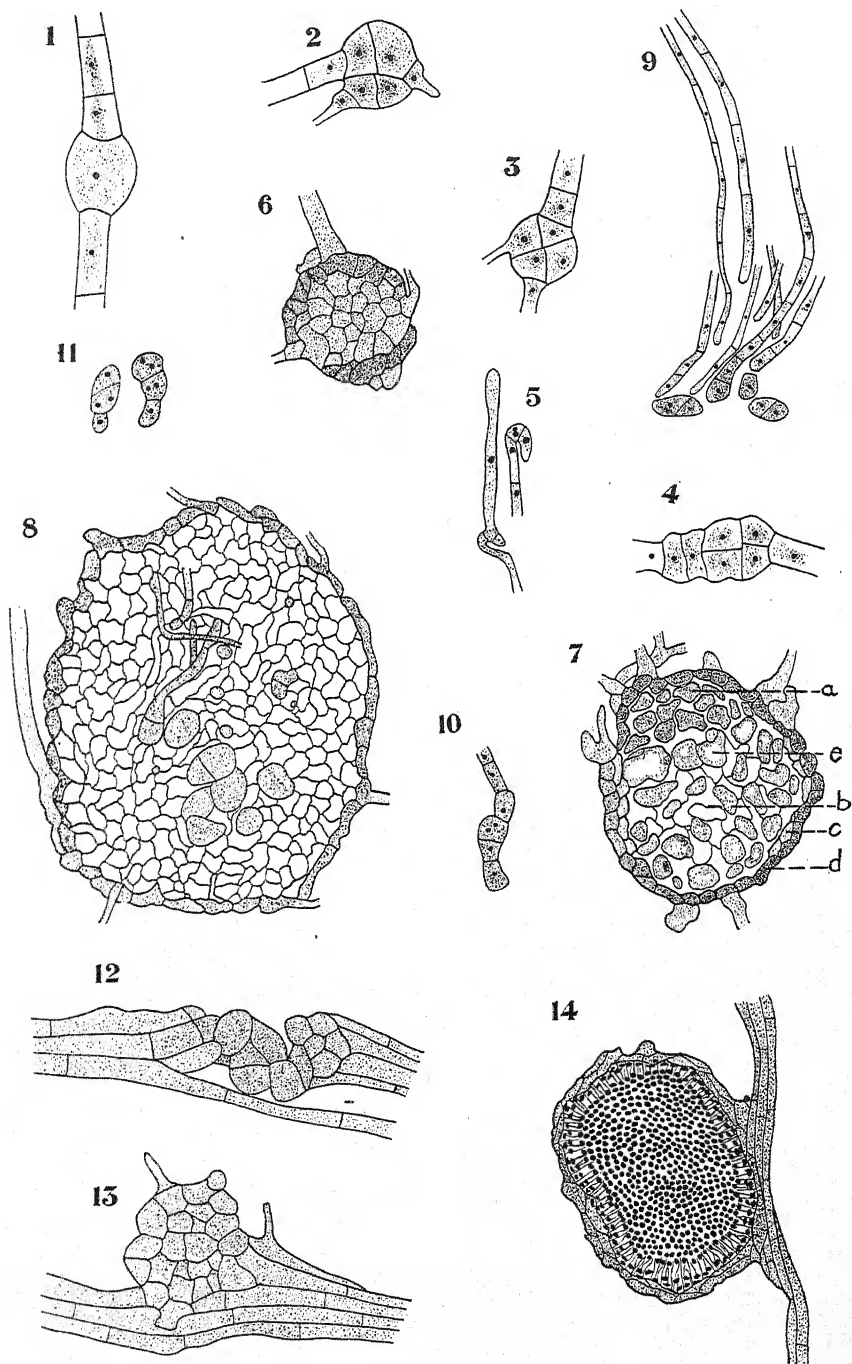
A culture of this fungus, No. 15922, has been deposited in the herbarium of the Department of Plant Pathology at Cornell University.

This work was carried out under the direction of Prof. H. M. Fitzpatrick, to whom the author wishes to express his appreciation.

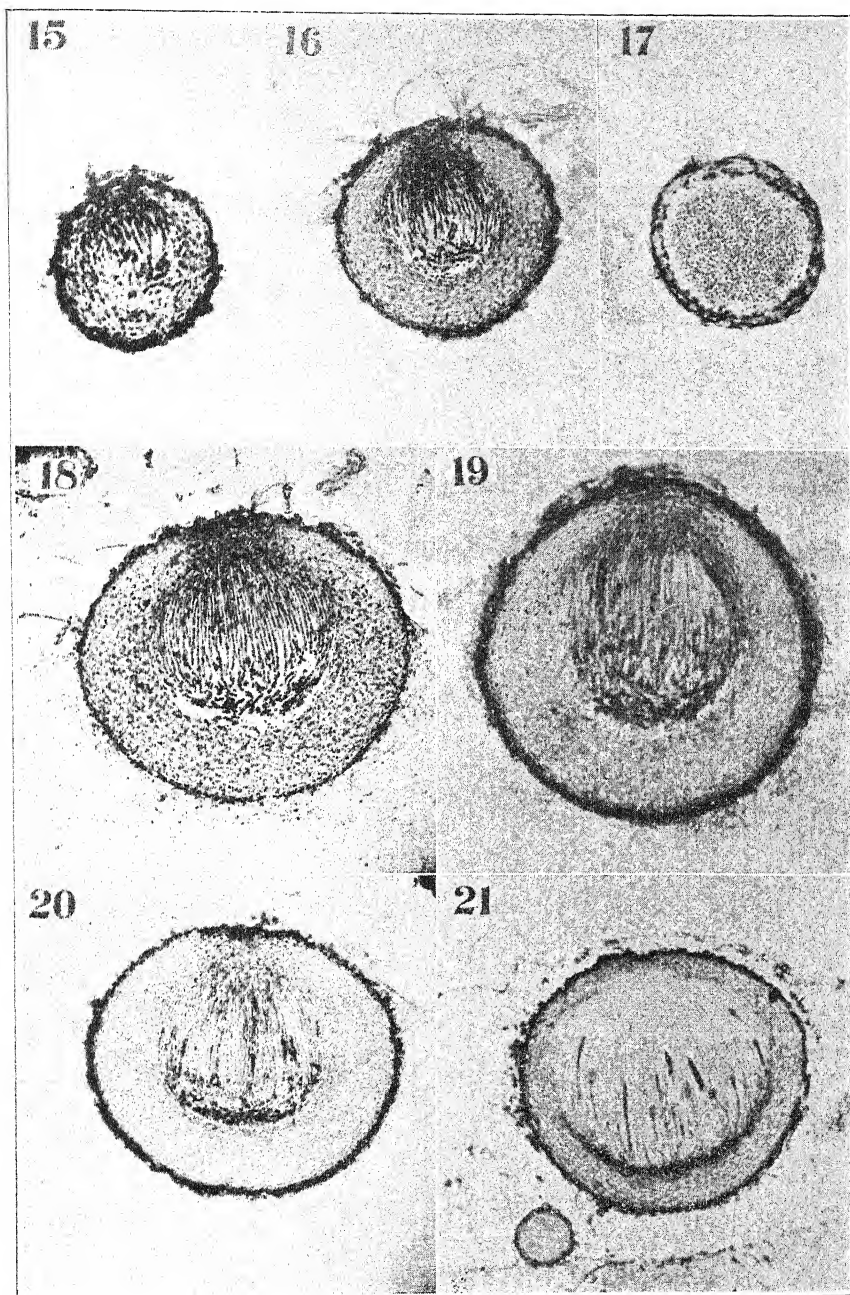
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LITERATURE CITED

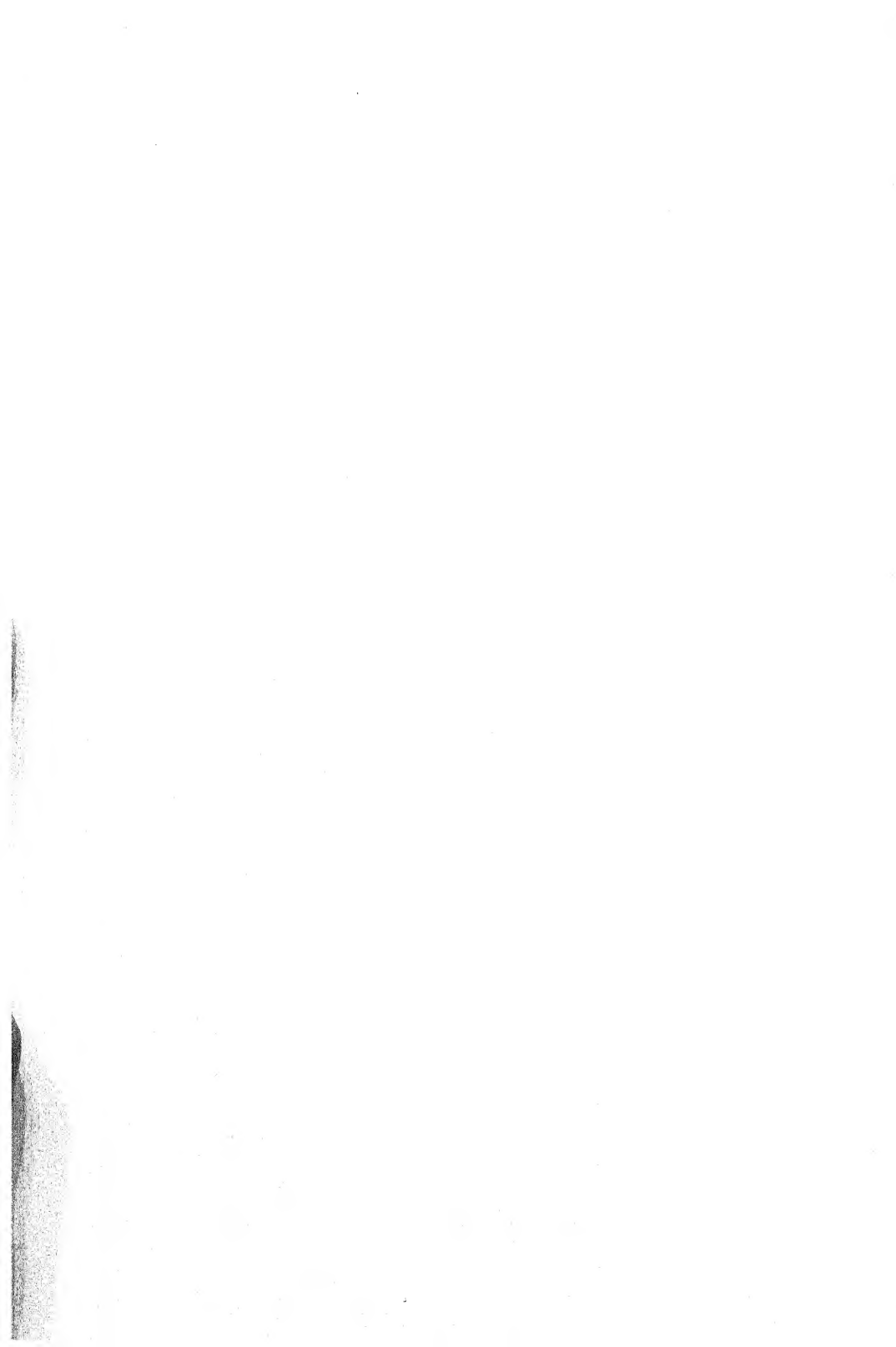
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ARNOLD: SPORORMIA



EXPLANATION OF PLATES

All sketches were outlined with a camera lucida.

PLATE XV

FIG. 1. A perithecial initial; a single, enlarged, uninucleate cell. $\times 690$.

FIGS. 2, 3, 4. Perithecial initials in which the first divisions have occurred. $\times 690$.

FIG. 5. Ascus hooks; one with a young ascus and the other with two nuclei in the penultimate cell. $\times 690$.

FIG. 6. Young perithecium after considerable enlargement has taken place. The outer layer is dark and the inner cells are thin walled and lightly stained. $\times 690$.

FIG. 7. More advanced stage showing beginning of internal differentiation. At *a*, compact, deeply stained cells near the top; *b*, cells of the interior where some loosening has taken place; *c*, discontinuous layer just beneath outer layer which will become the main part of the perithecial wall; *d*, brown outer layer; and *e*, enlarged cells, which, in later stages, appear at the tips of the vertical hyphae. $\times 690$.

FIG. 8. Young perithecium cut horizontal to its vertical axis and across its equatorial plane. The enlarged cells show attachment to young vertical hyphae. Slightly later than figure 7. $\times 690$.

FIG. 9. Vertical hyphae showing enlarged cells at their tips. All cells contain one nucleus. $\times 690$.

FIG. 10. Enlarged binucleate cell attached to the tip of a vertical hypha. $\times 690$.

FIG. 11. Enlarged binucleate cells which occur at the tips of vertical hyphae. $\times 690$.

FIG. 12. Rhizomorph with knot; a spermatogonial initial. $\times 690$.

FIG. 13. Spermatogonium showing further development. $\times 690$.

FIG. 14. Mature spermatogonium with spermatia and attached to rhizomorph. $\times 345$.
Semi-diagrammatic.

PLATE XVI

FIG. 15. Vertical section of young perithecium at about same stage as figure 8. The vertical hyphae extend into the cavity from the top. $\times 340$.

FIG. 16. Further developed stage showing enlarged cells at tips of vertical hyphae. $\times 240$.

FIG. 17. Mature spermatogonium with spermatia in a gelatinous mass in the interior. $\times 375$.

FIG. 18. Young perithecium showing increase in number of vertical hyphae and the zone of enlarged cells at their tips extending across the lower part of the cavity. $\times 222$.

FIG. 19. Young perithecium showing asci growing upward from the zone of enlarged cells at the tips of the vertical hyphae. $\times 255$.

FIG. 20. Same as figure 19 but slightly more mature. $\times 184$.

FIG. 21. Nearly mature perithecium. The enlarged cells are pressed firmly against the bottom of the cavity. Numerous asci also appear. $\times 150$.

PRELIMINARY STUDY OF THE NUTRITION OF THE CULTIVATED MUSHROOM

J. FRANKLIN STYER

(Received for publication October 16, 1927)

Mushrooms are commercially grown upon composts of horse manure. As the available supply of manure is becoming less, it is important to explain the wide difference between average and maximum yields. This requires a better knowledge of the nutrition of the cultivated mushroom than we now have.

The first attempts to culture *Agaricus campestris* L. in media of known composition were made by Duggar (2), who made a series of cultures on gray filter paper to which were added various carbonaceous and nitrogenous compounds, along with a mineral nutrient solution. His successful cultures included those containing calcium hippurate, peptone, and casein; while the unsuccessful ones were upon the common sugars, starch, mannite, tartrates, lactates, urea, asparagine, ammonium salts, and nitrates. He concluded that the fungus could not utilize carbon in the form of sugars and organic acid salts, nor nitrogen in inorganic form.

This conclusion is surprising in view of the general use of these compounds in culture media for fungi. Even the higher fungi have been grown upon such media, Lutz (6) having used a solution of xylose, maltose, ammonium salts, and minerals for hymenomycetes of several types. It is possible that Duggar's negative results were due in part to some adverse condition not easily observed rather than to the inability of the mycelium to develop upon simple nutrients. This possibility was considered in planning the present investigation.

Hébert and Heim (4) asserted that calcium and potassium should be added to manures used in the commercial culture of mushrooms, but supported the assertion only with analyses of mushrooms showing high contents of these elements. Later Hébert and Heim (5) reported that the composting of manure for mushroom culture was marked by losses in ammonia, organic acids, gums, cellulose, and xylan, and by increases in vasculose ("the black materials of the compost") and complex nitrogenous materials. They suggested that the nutrition of the mushroom was dependent upon a supply of the latter groups rather than of the former.

Boyer (1), in a study of *Agaricus campestris*, transferred his cultures from the original into manure, but said, "Almost all organic materials and many inorganic substrata are good." He did not obtain growth upon blotting paper or cellulose; however, it was not stated what medium was used with these materials. Boyer did not mention the inorganic materials used, except to state that potassium nitrate did not improve the growth in

any of his media. He does not appear to have been acquainted with Duggar's work.

EXPERIMENTAL RESULTS

Following the method of Duggar (2), filter paper was used as a base for the culture medium. Nine grams of shreds of a high grade paper, dyed with a direct cotton black, were placed in a 150 ml. flask, and 18 ml. of one of the nutrient solutions to be tested was added. Insoluble materials were stirred into the paper. The flasks were sterilized in the autoclave and inoculated in the center from a pure culture of spores of *Agaricus campestris*. The cultures were incubated at 25° C. upon pans of damp cotton, the latter used to keep the atmosphere moist while not tightly enclosed. Growth of the mycelium was recorded in tenths of the distance between the point of inoculation and the sides of the flask. Note was also made of the vigor of growth as evidenced by the density of the mycelium. The mycelium stands out plainly against the black paper. Duplicates were made in all cases.

The result of a series of cultures, using a number of sugars and other organic compounds, is shown in table I. Solution A was as follows:

MgSO ₄	0.02	molar
K ₂ SO ₄	0.01	"
KH ₂ PO ₄	0.04	"
CaCl ₂	0.002	"
FeSO ₄	trace	
NH ₄ NO ₃	0.1	"
NaOH to bring pH to 6.0		

TABLE I. *Effects of Some Carbonaceous Materials upon the Growth of Agaricus campestris. Growth Measured on a Basis of a Possible 10 at the End of 18 Days*

No.	Materials		Growth	Vigor
1.....	Paper, sol. A, dextrose	0.015 M	5	Good
2.....	" " "	0.05 M	5	Poor
3.....	" " "	0.40 M	1	Very poor
4.....	" " sucrose	0.007 M	6	Good
5.....	" " "	0.20 M	2	Very poor
6.....	" " mannite	0.015 M	5	Good
7.....	" " "	0.10 M	3	Poor
8.....	" " mannose	0.015 M	3	Good
9.....	" " "	0.10 M	2	Poor
10.....	" " maltose	0.007 M	4	Good
11.....	" " "	0.05 M	5	"
12.....	" " dextrine	0.007 M	5	"
13.....	" " "	0.05 M	7	"
14.....	" " Ca oxalate	0.045 M	7	Very good
15.....	" " "	0.30 M	7	" "
16.....	" " Na tartrate	0.03 M	4	" "
17.....	" " "	0.20 M	0	" "
18.....	" " K citrate	0.03 M	6	" "
19.....	" " "	0.20 M	0	" "
22.....	" " K lactate	0.06 M	7	" "
23.....	" " "	0.40 M	0	" "
24.....	" " starch	1 gm.	6	" "
25.....	" " (check)		7	" "
26.....	" distilled water only		1	Very poor

The fine growth of the check culture containing no substance except paper to provide energy suggests that the additions in the other cultures were superfluous and that cellulose plays an important part in the nutrition of this fungus. No evidence may be drawn of the value of the sugars and organic salts in this experiment, and the same should be true of Duggar's work (2) on such substances. The use of a relatively inert base is necessary.

Table 2 presents the result of a series of cultures with various sources of nitrogen. Each culture contained 0.33 percent each of dextrose, dextrine, and starch, along with solution B, which was the same as solution A but without ammonium nitrate.

TABLE 2. *Effect of Nitrogenous Materials upon the Growth of Agaricus campestris, Measured on the Basis of a Possible 10 at End of 18 Days.*
Cultures Contained Paper and Solution B

No.	Materials	Growth	Vigor
1.....	Glycine 0.1 M	8	Good
2.....	Asparagine 0.1 M	7	"
3.....	Pepsin 2%	10	Very good
4.....	Peptone (Witte) 2%	6	Good
5.....	Albumin 3%	9	Very good
6.....	Casein 3%	7	" "
7.....	Gelatin 2%	5	" "
8.....	Urea 0.1 M	4	Good
9.....	Hippuric acid 0.02 M	2	Very poor
10.....	NH ₄ NO ₃ 0.1 M	8	Very good
11.....	" 0.5 M	2	Poor
12.....	(NH ₄) ₂ SO ₄ 0.05 M	8	Good
13.....	(Check, no N)	3	Very poor

The cultures with peptone, gelatin, and urea were slow in starting growth, but at 18 days were growing even more vigorously than the others.

It seems certain that the range of possible sources of nitrogen for the organism is wide. Inorganic nitrogen in the form of ammonium compounds gave excellent growth, although the more complex forms gave slightly denser growth.

Table 3 presents the result of a series of cultures in which the partial concentrations of certain mineral salts were varied, along the general plan of the salt balance work of Haenseler (3). However, in the triangle method of comparing concentrations of three salts Haenseler used salts each containing two essential elements, which made it impossible to vary the concentration of individual elements. In this investigation the elements were considered separately, and after the elimination of calcium and nitrogen, both of which were studied separately, magnesium, potassium, sulphur, and phosphorus were chosen, and used in the form of their common radicals, Mg, K, SO₄, and H₂PO₄. As two of these are positive and two negative, by using the four possible compounds that they form it was possible to make a series of twenty-five cultures, each with a salt con-

centration of 0.02 *N*, and in which all possible partial concentrations of separate radicals in multiples of 0.005 *N* should be obtained. This series is represented in table 3 in the form of a square. Thus in any vertical column of cultures the concentration changes from 0.02 *N* sulphate with no phosphate, to 0.02 *N* phosphate with no sulphate, in increments of 0.005 *N*. At the same time, from left to right in any horizontal column the concentration changes from 0.02 *N* magnesium to 0.02 *N* potassium in the same manner. Vertical columns are designated K_0 to K_4 , after their potassium concentration, and horizontal columns P_0 to P_4 , after their phosphate concentration. The use of primary phosphates made possible the variation of partial concentrations on the normality basis, without too low concentrations of phosphate.

All the cultures contained 0.1 *M* NH_4NO_3 , 0.002 *M* CaCl_2 , FeSO_4 trace, and NaOH to bring the pH to 6.0, as did also the check culture lacking the four radicals to be studied. Thirty days were required for incubation as the temperature was lower than in former experiments.

TABLE 3. *Effect of Variation of Partial Concentration of Four Salt Radicals upon Growth of Agaricus campestris, Measured on the Basis of a Possible 10 at End of 30 Days. For Explanation see Text*

		(SULPHATE)						
		K_0	K_1	K_2	K_3	K_4		
(MAGNESIUM)	P_0	2	2	2	3	1	(POTASSIUM)	
	P_1	5	5	5	5	5		
	P_2	5	5	6	6	5		
	P_3	6	6	6	7	7		
	P_4	6	6	7	6	7		
		(PHOSPHATE)						
		check I						

The results as shown in table 3 indicate that magnesium, potassium, and sulphate sufficient for good growth may be supplied from the extremely small quantity in the filter paper. Analysis of the paper shows that of the four elements in this experiment it contains potassium in by far the greatest amount, about 0.07 percent; and this is equivalent to 0.005 *N* in the culture, about the same as the potassium concentration in the K_1 column. This is the smallest amount added to any of the cultures. And since a very small amount of the paper was attacked by the mycelium, the conclusion may be drawn that vigorous growth occurred where the concentrations of magnesium, potassium, and sulphate were each much below 0.005 *N*.

A similar series of cultures was made in which the total and partial

concentrations of the radicals were five times as great. Growth was vigorous in the entire series except those cultures lacking phosphate, showing that 0.1 *N* concentration of each of these radicals is not too high.

SUMMARY

1. *Agaricus campestris* when grown upon a paper medium, with mineral nutrients and an ammonium salt, required the addition of no other carbonaceous material.

2. The addition of various sugars and salts of organic acids gave no greater vigor of growth, and resulted in a lack of vigor when above about 0.05 Molar concentration.

3. Ammonium salts, urea, glycine and asparagine, peptone and proteins are all useful as sources of nitrogen, the most complex being slightly the most effective.

4. Vigorous growth was obtained when the phosphate concentration was between 0.015 Molar and 0.1 Molar; and when the sulphate, potassium, and magnesium concentrations were each between 0.005 Normal and 0.1 Normal. These, with the exception of the lower figure for phosphate, are not definite limits, and the organism can probably tolerate concentrations outside these limits.

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CARPEL MORPHOLOGY IN THE CRUCIFERAE

ARTHUR J. EAMES AND CARL L. WILSON

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the investigation of the floral anatomy of the Cruciferae and some families, with the object of determining the fundamental floral structure of the groups, the writers have obtained evidence which compels us to reopen a subject of long controversy—the nature of the ovary of cruciferae. Further, light is also shed upon the recently proposed theory of carpel polymorphism as applied to this family.

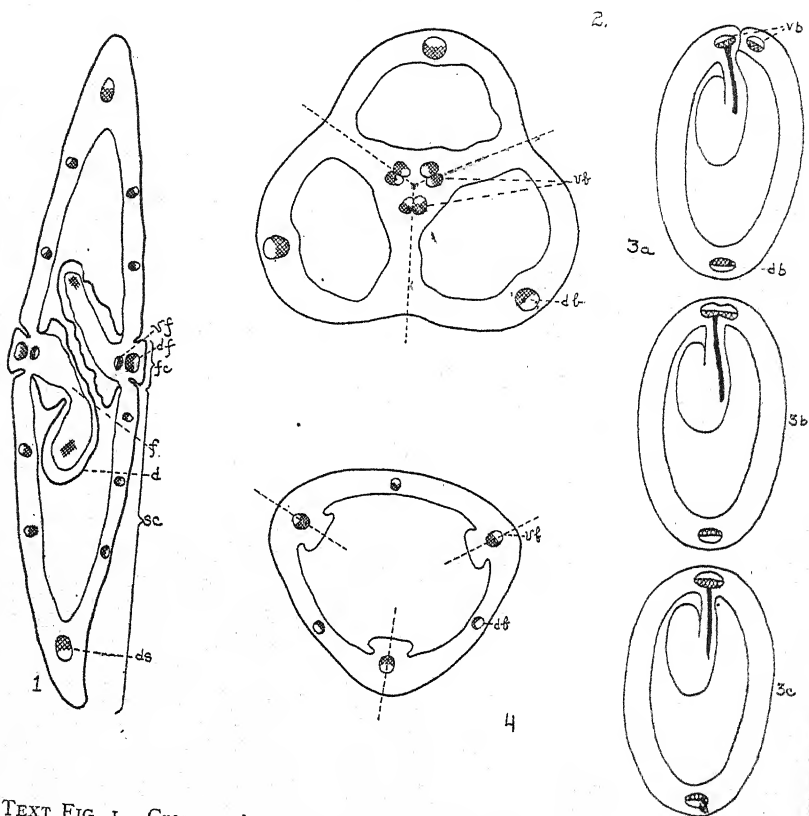
The structure of the crucifer gynoecium has long been a subject of controversy, especially in regard to the number and type of carpels, and the position of the septum, or false partition. Students of this subject have advanced several theories as to the nature of the ovary. Of these the chief are, (1) that the pistil consists of two carpels; (2) that there are four carpels, in one whorl or in two whorls of two each. That the number of carpels is six has also been maintained; and recently as many as 10–20 carpels have been considered to compose the pistil in some genera.

The two-carpel theory seems to be commonly held today. According to this understanding there are two open carpels fused edge to edge forming the ovary. The fused margins of these carpels bear the placentae, and outgrowths of the placentae divide the really one-celled ovary. The septum is *false*.

Those students holding that there are four carpels have generally believed that two of these are reduced or aborted. In such cases the ovules are borne on placental surfaces (of which there are four), placed between the normal and the reduced carpels; and opinions have varied as to the carpel to which the ovules belong, and hence which type of carpel is sterile and which is fertile. A prominent theory, put forth and emphasized by older writers, and recently renewed, and supported with new facts by Saunders (26), is that the so-called valves of the ovary make up the four carpels, the fertile carpels being composed of the segments between the valves. The relationship of these two types of carpels can be understood by reference to text figure 1.

Many supporters of this theory, as well as of the two-carpel theory, have considered the septum, or false partition, to be a secondary placental growth. A few have considered this dissepiment to represent carpellary tissue of the fertile carpels. If the septum is truly carpellary, then it must represent either the ventral part of the carpel itself or a much extended margin of the carpel. If the dissepiment is false, that is, of placental

origin, and not morphologically formed from the carpel wall, then the placentae are normal parietal placentae, and the ovules are borne in a loculus falsely divided into two chambers. Anatomical evidence, already used by earlier students, and again brought forward by the writers, shows



TEXT FIG. 1. Cross section of ovary of *Lepidium virginicum* showing *fc*, the fertile carpels, and *sc*, the sterile carpels; *df*, dorsal bundle of fertile carpel, and *vf*, the fused ventral bundles of fertile carpel; *ds*, dorsal bundle of sterile carpel; *f*, funicle of ovule; enclosed by *d*, dissepiment. TEXT FIG. 2. Cross section of ovary of *Trillium*, showing the vascular supply and carpel limits; *db*, the dorsal bundle, and *vb*, the inverted ventral bundles of one carpel. TEXT FIG. 3. The vascular supply of simple pistils, the ventral bundles, *vb*, inverted and free or fused; *db*, the dorsal bundle. TEXT FIG. 4. Cross section of ovary of *Viola*, showing the vascular supply and carpel limits; *db*, dorsal bundle, *v*, bundle resulting from fusion of ventrals of adjacent carpels.

that the ovules are not attached to the placentae in the manner in which ovules are normally borne on parietal placentae. The placentae are clearly not parietal and the above interpretations are not correct.

If the septum is not a placental outgrowth, and really is made up of parts of the carpels constituting the fertile pair, the ovules must the

lie in the chambers formed by the sterile carpels, an anomalous condition, since the ovules are attached to the vascular bundles of the fertile carpels. Although such a condition is remarkable, the writers believe it to be the true state, and submit anatomical evidence in support of the belief. They follow Miss Saunders and earlier students in believing that the fertile carpels are reduced or abortive,—have in a sense become “solid,”—and that the dissepiment represents the ventral parts, or margins of these carpels.

The fact that, if this is true, the position of the ovules must be explained, seems to have been overlooked, so far as the writers know, by students of the structure of the crucifer ovary. Only Rendle (25) in outlining Miss Saunders' theory in his taxonomic synopsis of the family has called attention to this difficulty—“the position of the ovules at the outer edges of the septum (which is called a part of the carpel bearing the ovules) presents a serious difficulty which is not explained by the author.” The ovules can only have attained a position in the loculus of an adjacent carpel by pushing out through the wall of the carpels bearing them. This the authors believe to have happened.

NATURE OF THE OVARY

The opinion held by the writers as to the morphology of the crucifer pistil is supported chiefly by vascular anatomy. The vascular supply to the ovary is very simple and is in no way fundamentally unlike that of syncarpous ovaries in general. Normally, in the majority of angiosperm families, a carpel receives three traces,—a dorsal, or midrib bundle, and two ventral or marginal bundles. (The number in a few families is doubtless more, and in the case of reduced pistils, the three bundles are clearly reduced to one.) In the case of folded (closed) carpels, the ventrals usually become inversely oriented soon after they depart from the receptacular stele because of the up-folding of the carpellary leaf and the in-turning of the edges. In a syncarpous ovary composed of closed carpels there are present, therefore (text fig. 2), a ring of bundles (the midrib, or dorsal) in the outer ovary wall, normally oriented, and another ring (the lateral, marginal, or ventral), inversely oriented, in the center. The ring of ventrals typically contains twice as many bundles as does the ring of dorsals, but since the ventrals often fuse in pairs—those of contiguous edges of adjacent carpels uniting—there may be the same number of “ventrals” as dorsals. These pairs of bundles may only be slightly fused, forming double bundles, or may show any stage of fusion up to such complete union that no histological evidence of double nature exists. Where the pistil consists of a single carpel the ventrals may remain separate or show any degree of fusion. Thus such a pistil, seen in cross section, will have two or three bundles (text fig. 3); if two, the ventral bundle represents two fused marginal strands.

In ~~cases~~ ^{the ovary} is composed of open carpels—with the margins of

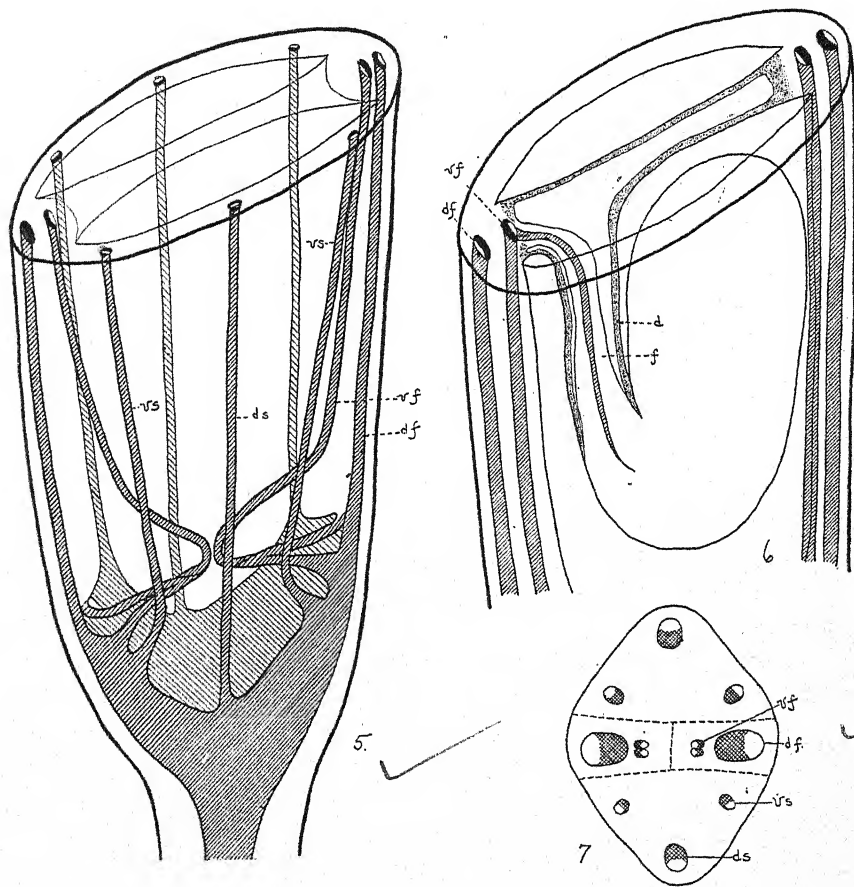
adjacent carpels fused, each individual carpel remaining open—the ventrals are not inverted since the edges of the carpel have not been infolded. Thus, in figure 4, the bundles of the ovary wall are all normally oriented, alternate bundles being dorsals and ventrals. In ovaries of this type the contiguous ventrals of two adjacent carpels may likewise become fused to form a single bundle (as in the figure), often clearly of double nature. Thus the structure of ovaries varies in the orientation of bundles, and these variations depend, at least in part, upon the primitively open or closed state of the carpels composing them.

The vascular anatomy of the crucifer pistil follows this uniform structural scheme. However, whereas syncarpous ovaries are commonly made up of either of fused closed carpels or of fused open carpels, the ovary of the crucifers is composed of both closed and open carpels—two inner closed carpels partly surrounded and enclosed by a lower, outer whorl of two open carpels, a very unusual structural condition which has made puzzling the nature of the crucifer ovary. That this is the true morphological condition is demonstrated by the number, course, and orientation of the carpellary traces.

In the receptacle of the flower, after the traces to the sepals, petals, and stamens have passed out, there remains a cylinder of vascular tissue (text fig. 5). From this cylinder there first pass out traces to the sterile carpels. At a slightly higher level the traces to the fertile carpels pass off. In both instances the vascular supply of a carpel consists of the usual three bundles, a dorsal or midrib trace, and two ventral or marginal traces. The dorsal traces retain their normal orientation, as do the ventral traces of the open sterile carpels. The ventral traces of the closed fertile carpels, on the other hand, become inversely oriented. Cross sections of the receptacle at the level of the passing off of the traces to the fertile carpel reveal the manner in which this change is brought about. As the bundles, at first normally oriented, pass in, the position of the phloem and xylem is gradually reversed. In many species, these two bundles fuse, and the bundle so formed approaches the similar bundle of the other carpel in the center of the ovary; the bundles then swing back toward the dorsal bundles (text fig. 5) and take up a position just inside the dorsals. These ventral bundles, double in origin, accompany the dorsal bundles to the top of the ovary, giving off traces to ovules as they proceed (text fig. 6).

The ventrals of the fertile carpels do not always behave in the manner shown in figure 5. They may not fuse, but approach the center of the ovary and then retreat to the position just inside of the dorsal, meanwhile remaining distinct. Again, all four such traces may meet in the center and form a somewhat confused mass of vascular tissue, in which the component traces are distinguishable with difficulty. In other cases the ventrals of each carpel fuse without passing far toward the center of the ovary, and then swing slightly backward and follow up inside the dorsals. In a

few species the ventrals, after fusing near the center of the ovary, split apart again and swing back, taking position to the right and left inside of the dorsals. Thus there may be for each fertile carpel either one or two



TEXT FIG. 5. Diagram of base of crucifer ovary showing vascular skeleton, especially the origin and course of the inversely oriented ventral bundles, *vf*, of the fertile carpels; *df*, dorsal bundle of fertile carpel; *ds* and *vs*, dorsal and ventral bundles of sterile carpel, respectively. TEXT FIG. 6. Diagram of part of crucifer ovary showing relation of ovule to septum; the ovular trace is derived from the inverted ventral bundle of the fertile carpel; the funiculus lies loosely within the septum (here shown as though sectioned to the chalazal region); *df* and *vf*, dorsal and ventral bundles, respectively, of the fertile carpel; *f*, funicle, *d*, dissepiment. TEXT FIG. 7. Diagram of cross section of base of ovary of *Cardamine Douglasii* showing vascular supply to the carpels (labelling as in figure 5) and carpel limits (indicated by dotted lines).

bundles representing the ventral traces which pass up the ovary wall. These bundles always lie to the inside of the dorsals, always arise as two bundles after the dorsal has arisen, and are *always inverted*. These facts,

together with their passage to the center of the ovary as soon as they are formed (the normal behavior of ventrals in cases of *axillary* placentation), makes it certain that these are normal ventrals. Their position close to the dorsals can only be a derived one; the retreat from the central position in the base of the ovary shows how this position has been attained.

The outer whorl of two carpels also receives six traces, two dorsals and four ventrals, which arise in the position and sequence normal for the traces to a whorl of two carpels—first the two dorsals, then the ventrals, one on each side of and above each dorsal (text fig. 5). These ventrals do not become inverted but pass directly into the margins of the valves of the ovary—the normal position and course for such ventrals in an open carpel. If ovules were borne by these carpels, their bundle traces would be derived from these ventral bundles. The fact that these ventral traces arise far around the stele laterally, and appear, unless carefully studied, to be derived from the dorsal trace to the fertile carpels, was disconcerting to Hannig (11) and Gerber (9), and led them to believe that these marginal bundles of the sterile carpels really belong to the fertile carpels, thus greatly confusing their attempts at interpretation. These traces are, however, normal in place and method of origin; they arise from the stele far around laterally, and the remaining parts of the stele become the traces to its terminal appendages, the two fertile carpels.

The six carpel traces last formed are those normal in number, position, and orientation for two closed carpels—two dorsals passing directly upward, and four ventrals which swing inward to the center of the ovary, becoming inverted as they do so. It is the behavior of these four ventrals which is of the greatest importance to the interpretation of the ovary structure. A section at the very base of the ovary (just below the loculi) shows a structural condition for the two fertile carpels which is the same as that of any two normal folded carpels (text fig. 7)—two dorsals and four inverted ventrals. (These ventrals in some species are fused with one another to a greater or less extent.) The carpels are obviously normal in anatomy at the base; if they were normal throughout, the ventral bundles would pass (as they commonly do among angiosperms) directly upward through the central part of the ovary. However, in these plants they swing outward and pass up just inside the dorsals, retaining their inversion. In spite of this peculiar behavior, however, it is clear from the number, orientation, and original course of these traces that they are the true ventrals of an upper set of two carpels. The outward swinging is obviously due to change of course resulting from a modification of the carpels. This modification is a strong reduction, which has apparently taken place by contraction, the loculus being obliterated, and the carpel becoming "solid."

Perhaps the very unusual relationship of the lower carpels to this upper pair—a nearly complete enclosure—has been the factor determining in large degree the reduction of the upper whorl. The remarkable fact

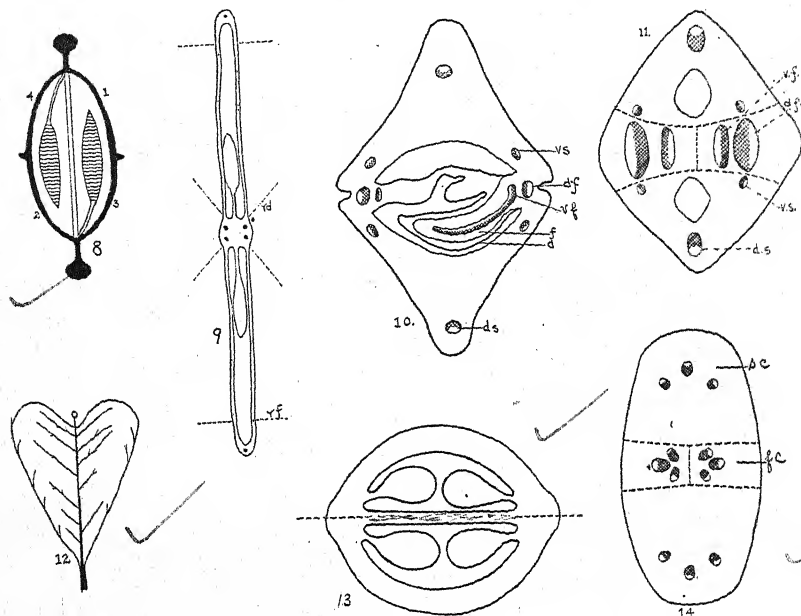
is that it is the reduced carpels which have remained fertile, whereas the well-developed ones are sterile.

On the basis of this interpretation of the carpel number and position, the writers believe all structural conditions are explained. The theory that there are four carpels in two whorls of two each, two of the carpels being abortive, was doubtless first suggested by Lindley (19). Lindley's view has been followed, more or less closely, by Kunth (according to Lindley and Duchartre), Godron (10), Klein (15), Martel (20), Hannig (11), Kerner and Oliver (14), Henslow (12), Miss Saunders (25), and others.

Among these students, opinions as to position and limits of carpels have varied greatly, and the septum has been given several interpretations. These modifications of the four-carpel theory need not be discussed in detail here, since Gerber and Miss Saunders have reviewed the history of opinion on this subject. An outline is, however, desirable. In the opinion of the majority of those holding this view, the narrow commissural segments represent two carpels, the broad valves the other two. Henslow's (13) view, however, was that all four carpels are equally developed, the commissural lines representing two lines of marginal fusion, and the middle lines of the valves the other two (text fig. 8); the latter two lines of fusion, which would normally bear placentae, are sterile. According to Gerber (9), there are, in addition to the two valve and the two placental carpels, two internal carpels which form the dissepiment. These two carpels are entirely enclosed by the other four, and stand back to back with their ventral edges outward, a position the reverse of that of normal carpels. This idea of enclosure of carpels entirely within other carpels (*i.e.*, normally—it occasionally happens abnormally), and especially the complete reversal of orientation of some of the carpels of a flower (their dorsal edges inward), is rather too fantastic for acceptance. Gerber, however, was led to this conclusion by the important discovery of the inverted ventrals. To him the inversion of the marginal bundles could only be explained by the inversion of an entire carpel. This investigator thus believed the cruciferous ovary to consist of six carpels (his opinion as to the four others being the same as that of most other holders of the four-carpel theory). Hannig (11) also investigated the inverted bundles in the Cruciferae, substantiating Gerber's discovery of the inversion, and extending it to many other genera. He determined the origin of these bundles, which had not been investigated by Gerber. However, like Gerber, he is able to place no other interpretation upon the inverted bundles than that they are evidence of inverted carpels. Therefore, it was the opinion of these men that the septum is distinctly carpellary in nature, but represents two entire carpels quite distinct from the four usually seen.

Chodat and Lendner (5), according to Gerber, were convinced of the four-carpel nature of the ovary, but held a markedly different opinion as to the nature of the septum. This and other theories concerning the septum are discussed below.

A brief consideration of the two-carpel theory may not be out of place since it is so commonly held today. This was emphasized by De Candolle (4), and followed by Schleiden (29), Payer (22), Eichler (7), Lignier (17), Van Tieghem (31), and most recent students. Figure 13 shows diagrammatically the common 2-carpel interpretation. The chief difficulty en-



TEXT FIG. 8. Cross section of a cruciferous ovary. The numbers, 1-4, represent the position of four carpels making up the ovary according to the theory of Henslow (after Henslow). TEXT FIG. 9. Cross section of a cruciferous ovary; *rd*, region of dehiscence of valves; *rf*, region in which, according to Miss Saunders, fusion of sterile and fertile valves occurs (after Miss Saunders). TEXT FIG. 10. Diagram of cross section of ovary of *Brassica*, showing vascular supply of the four carpels and the position of the funicle, *f*, (cut obliquely longitudinally) within the septum, *d*; *ds* and *vs*, *df* and *vf*, dorsal and ventral bundles of sterile and fertile carpels, respectively. TEXT FIG. 11. Diagram of cross section of base of ovary of *Polanisia graveolens*, showing the carpel limits (dotted line), and the vascular supply to the four carpels; *vf*, the pairs of inverted ventrals of the fertile carpels (labelling as in figure 10). TEXT FIG. 12. Silicle of *Capsella Bursa-pastoris* (after Miss Saunders). TEXT FIG. 13. Diagram of crucifer ovary showing the carpel limits according to the 2-carpel theory, the septum being an outgrowth of parietal placentae. TEXT FIG. 14. Diagram of cross section of base of ovary of *Dicentra spectabilis*, showing the carpel limits (dotted lines), and the typical 3-bundle supply for each carpel; the ventral bundles of the fertile carpels, *fc*, only partly inverted. (*Sc*, sterile carpel.)

countered in this interpretation has always been that in most genera the stigmas are situated over the commissures and not over the midribs of the carpels, the normal position for stigmas. The method of dehiscence, apparently neither loculicidal nor septicidal, is on this interpretation also difficult to explain. (See later discussion of these points.)

De Candolle believed the two carpels to be closed, not open as most others have thought, their ventral parts forming the septum. On this basis the same difficulties of stigma-position and dehiscence are present.

When the anatomy is considered, however, all theories that two carpels only are present must be discarded; and apparently no one who has followed the course of the traces to the floral organs has believed as few as two carpels to be present. De Candolle's theory must be discarded because of the lack of ventral traces which should be present in the center of the septum, and because the ovules would, on this interpretation, be borne midway on the side of the carpels. That the septum can not be merely a placental outgrowth is obvious from the presence of the inverted bundles in its margin. The two-carpel theory clearly must be discarded for the four-carpel theory.

NATURE OF THE SEPTUM

Wrapped up in both these theories, and often necessarily involved, is the question of the nature of the septum. This structure demands some description. It consists of a thin, often delicate partition stretched between the commissural framework, the replum.¹ At low levels in the ovary, the septum may be a thick, solid structure, extending from one fertile carpel to another and separating the loculi of the two sterile carpels. Higher up in the ovary, it appears hollow and double (text fig. 6), made up of two thin walls lying side by side, each wall being formed of a single layer of cells. The space between these walls is in some cases filled with a loose, mesophyll-like, photosynthetic tissue; in others the space is empty. In many forms the septum is made up of two parts, one part connected with each fertile carpel (text fig. 1). Often the septum parts are united in either the upper or lower parts of the ovary.

As already stated, this partition is commonly looked upon by exponents of both these theories as a median placental outgrowth of secondary nature. The presence in the septum of the inverted bundles—the "inner replum bundles" of Gerber and Hannig—is, however, sufficient proof that this is not the case. Such outgrowths are without bundles, or at least possess no fundamental vascular system.

If the septum is not placental, it must be carpellary. This view has been held by many students, and there are nearly as many opinions as to the part of the carpel involved as there have been students of the subject. De Candolle believed that two carpels were fused together as in a normal syncarpous ovary with axial placentation. The septum then would be made up of the fused ventral sections of the two carpels. But as above stated, the absence of bundles where strong ventrals should be and the presence of bundles where none should be, were this the case, is sufficient proof of the incorrectness of this view.

¹ The term *replum* is also sometimes applied to this framework plus the septum, but the two terms are probably better applied to separate parts. The terms *dissepiment* and *false partition* are also applied to the septum.

Gerber believed the partition, that is, the part between the placentae proper, to represent entire carpels, inverted (see above). Yet such carpels would be without dorsal bundles, for bundles have not been found in the middle of the septum by any investigator. Fournier (8) also held this view of two median carpels, back to back. He apparently held, however, that the carpels were not entirely enclosed by the valves, as Gerber believed, but that the ventral edges were exposed and formed the suture segments. Again the absence of dorsals where dorsals should be, and the presence of two series of bundles in the marginal region, disproves this theory. Further, this theory, together with that of Gerber, demands the absurd condition of two whorls of carpels, one standing with dorsal margin outward, the other with ventral outward.

Chodat and Lendner have believed the septum (or its inner portion) axillary in nature, that is, to be the receptacle prolonged and flattened. The fact that it contains no stele and no evidence of central vascular tissue, even of vestigial nature, should be sufficient proof that the axis is not here prolonged to the top of the ovary. As further proof, the double nature of the septum may be offered. This two-layered condition is often noticeable in the ripe fruit, and it is particularly prominent in sections of the ovary and developing fruit. Still further, in ontogeny the septum is built up by the centripetal growth of the developing anterior-posterior carpel primordia. No axial structure is formed in this manner.

Evidence from ontogeny is the basis for the theory of placental outgrowth in explanation of the nature of the septum. Such evidence is also used by exponents of the theory that the septum represents the expanded or developed ventral margins of the carpels. The method of development has been shown by Payer (22) and others. It is clear that the two fertile carpels develop much as do the carpels of a normal syncarpous ovary with closed carpels. Ontogeny certainly suggests that the septum is carpellary in nature. Anatomy and histology bear out this contention. The vascular supply is that of carpels; the ventrals, however, are close to the dorsals, indicating by their position a reduction or contraction such that the loculus has (wholly or nearly) disappeared, forming a sort of "solid" carpel. This being the case (and the course of the bundles in the base of the ovary is strong proof thereof), on this basis the part lying inward from the inverted bundles must represent the narrow strip of tissue on the ventral margin of the folded carpel. This strip in these cases is much extended and forms a broad wing on the reduced carpel. The wings of the two carpels meet in the center and the septum is formed.

The outer cell-layers of the septum are remarkably epidermis-like, and Trécul (30) has shown that they are provided (in several genera at least) with stomates. Hannig (11) has found the septum to be provided with a cuticle also. The authors have studied the presence of stomates and believe that these and the presence of the cuticle are further strong proof

of the carpellary nature of the septum. The following forms were found to bear stomates on the dissepiment: *Brassica oleracea* L., *B. nigra* (L.) Koch, *B. juncea* (L.) Cosson, *B. arvensis* (L.) Ktze., *Cheiranthus Allionii*, Hort., *Barbarea vulgaris* R. Br., *Sisymbrium altissimum* L., *Capsella Bursa-pastoris* (L.) Medic., *Lepidium campestre* (L.) R. Br., *Erucastrum gallicum* (Willd.) Schulz., and *Raphanus sativus* L. Several of these also bore stomates on the funiculus. Payson (23) illustrates stomates on the septum in *Lesquerella densiflora* (Gray) Wats. To be sure, the stomates, as found by the authors, are largely restricted to the marginal regions of the septum—only rather rarely do they occur in the middle regions—but this distribution is that to be expected if the septum represents merely the expanded margins, regions which would normally be largely lacking in stomates. These stomates on the septum are normal in every way, giving still further evidence that the upper set of carpels has become enclosed by the lower set; and since the stomates are normal, and not vestigial, the enclosure has perhaps been comparatively recent.

Henslow (13) also considered the septum to represent the extended margin of carpels, and cited Duchartre's (6) figures of abnormal carpel-stamens in proof. Such structures are certainly suggestive of the origin of the septum from the fertile carpels. It is not clear, however, from Henslow's paper, just what he believed to be the relation of the septum to the four equally developed carpels (see above).

THE POSITION OF THE OVULES

The chief problem involved in the interpretation of the ovary as consisting of four carpels, two sterile and valve-like, and two fertile but reduced, is that of the attachment of the ovules. If the reduced fertile carpels be considered as reduced to their dorsal bundles (as suggested by Henslow, Miss Saunders, and others), with the ovules borne upon these naked remnants of carpels, little difficulty is involved—unless it be to explain what part of such carpels the septal projection represents. But the presence, position, and orientation of the ventral bundles precludes such an interpretation, and also the view that such a carpel is an open one of extremely narrow nature with the ovules borne normally upon its margins which lie close together because of its narrowness. The carpel must be a closed one, and since the loculus is lacking, it may in a sense be called a "solid" carpel. But what then of the ovules—how can ovules borne by a carpel lie outside that carpel and within the loculi of other carpels? The only answer is that their position must be a false, or derived one. That this is the case is clear on structural evidence. The ovular traces are derived from the inverted bundles (this is the typical origin from ventral bundles) and lead through long funicles to the ovules. These funicles pass out *through the septum* (text figs. 1, 6, and 10)—evidence in itself that the ovules belong within the carpels. The septum wall cloaks, usually very loosely, the

bases of the funicles and may in some cases be clearly seen over the bases of the ovules. It is thus evident that the ovules have "pushed out" from the "solid" carpels, which bear them, and lie outside the carpel body, in evaginated folds of the septum wall. Perhaps, in many cases, they have in the evagination "broken through" these walls, leaving only their funicles still sheathed by the walls.

The funicles are nearly always loosely surrounded by the projecting pockets of the septum wall (and these pockets are obviously evaginations of the wall as cross sections of the ovary show), but the ovules show no such loose cloak. In a few cases among the many species studied by the authors an outer layer continuous with the funicle sheath was sufficiently distinct to be considered as doubtless representing the septum wall about the ovule. *Brassica nigra* shows this more clearly than other species seen by the writers. In those species in which this coat over the ovule is not obvious, the coat has apparently either become an integral part of the integument (in which case of course the ovule has not broken through, but morphologically still lies within the "solid" carpel), or the ovule actually projects through the evaginated septum wall so far as its base, and lies naked in the loculus of the sterile carpel. The presence in several species of a chalazal 'shoulder' is suggestive of this condition.

That there is an unusual seed-coat condition in the crucifers is evident from occasional statements met in literature, such as that of De Candolle (4) "*Spermoderma crassiusculum*, extus ut videtur pellicula cinctum nunc adpressissima, nunc in alam membranaceam expansa . . .," "Seed coat rather thick, surrounded as it were by a pellicle which is sometimes very closely appressed and sometimes expanded into a membranaceous wing." It is of course not wholly clear that this "pellicle" represents the septum wall about the ovary, but this seems probable. And the fact that there is in taxonomic literature frequent mention of peculiar integument structure, extra seed coats, "three ovular integuments," etc., lends strength to the theory. The writers believe that this wall is at least in some genera present over the ovule, but that it has become, so far as the ovule is concerned, so closely appressed to and fused with the integuments that it is no longer evident as an extra layer.

That the septum wall extends at least over the funicles is clear from its obvious presence as a loose sheath as seen in sections (text figs. 1, 6, and 10), and from the fact that this sheath is not only continuous with the rest of the septum wall but is like it histologically and shows stomates as does the septum. (Efforts to find stomates upon the ovules were unavailing.) Still further proof that the funicles extend inside of the septum is found in the frequently used taxonomic character "funicles adherent to the septum." The funicles are not truly adherent (externally adnate), but they pass within the septum for a greater or less distance and are hence apparently adherent. The often noted persistence of the seeds attached to the replum

after the valves have fallen is doubtless due to this very enclosure of the funicles within the tissue of the septum.

It thus appears that there is in the crucifer flower the remarkable condition of ovules placed outside of (or seemingly outside of) the carpels which bear them. There is then this peculiar structural condition in addition to those already mentioned: an ovary of two kinds of carpels, one open, one closed; one kind abortive and specialized, yet still fertile; the other sterile and well developed, serving to protect in large measure the ovules borne by the reduced carpels.

DISCUSSION

Few subjects in flower morphology have attracted the attention of more students than that of the structure of the crucifer flower, especially the nature of the androecium and the gynoecium. Papers published on this subject are numerous, and the writers have been loath to add yet another. The theory that there are four carpels in the pistil is by no means a recent one; nor is the viewpoint that the replum and septum together represent reduced fertile carpels to be looked upon as new. Further, even the anatomical method in the solution of this puzzle is old;—Fournier (8) used it in his study in 1864. The authors claim only that anatomical evidence has now placed this interpretation on a firm basis. They desire to call attention particularly to the importance of the inverted ventral bundles in the interpretation of ovary morphology. Among the earlier papers dealing with the anatomy of the crucifer ovary, the works of Gerber (9), Hannig (11), Henslow (13), and Van Tieghem (32) stand out, and all of these deal with the inverted bundles, the first two emphasizing these unusual bundles as of the greatest importance in the interpretation of the ovary. The meaning of inverted ovary bundles was clear to Van Tieghem (31) and to Henslow (12), and was demonstrated in their works, which are classic for flower anatomy. The matter of inverted bundles seems, however, to have been overlooked in recent studies of this subject. In Miss Saunders' thorough morphological study the ventral bundles of the solid carpels are shown (26, fig. 11, in *Matthiola*), but the arrangement of xylem and phloem is not noted, nor is any particular significance attached to the presence of these bundles.

This double ("parallel") bundle system—the dorsal plus the ventrals—of the solid carpel is explained by Miss Saunders as "connected with the considerable width of the commissure, for by this arrangement the vascular bundle of the funicle has less distance to traverse before making contact than is the case when a single median bundle is present." "Where the suture is considerably narrower a single central bundle appears to be the rule"; it is difficult to see how *width* of the commissure influences the presence of extra, *radially-placed* bundles. Apparently the two or three bundles of the region are looked upon merely as part of one, but the inversion of the inner

ones would show this not to be the case even if some other satisfactory explanation were not available. The double bundle condition is considered by Miss Saunders to be more or less characteristic of solid carpels in general (outside of the Cruciferae), yet in most cases of such double bundles which she shows the two strands lie tangentially side by side, not radially. Tangentially double bundles represent an entirely different situation.

The authors are in full accord with Miss Saunders that there are in the crucifer ovary two whorls of two carpels each; that two of these are valve-like and sterile, the other two "solid" and fertile. They agree with her that these carpels are polymorphic only in a limited sense. Fundamentally they are alike—and like all other carpels in the angiosperms. She herself notes the similarity of vascular supply of the commissural region (the solid carpel) and that of the valves, stating that there is "in the smaller arc a vascular supply quite equal to that of the valve, but compressed into a smaller area, the bundles lying on a small circle instead of an extended arc." This is exactly the normal condition of the vascular supplies of the two carpel types; the difference is that of the closed (solid) carpel as against an open (valve) carpel. It can readily be seen that the closing of the open carpel by infolding of its edges produces the condition found in the solid carpel. The ventral bundles become inverted; only in the obliteration of the locus is the difference extreme.

In the matter of semi-solid carpels the authors are not in accord with Miss Saunders. They believe that no such carpel type—intermediate between the other two or of different nature—exists in this family. In their opinion silique and silicle are morphologically equivalent; the silicle (*silicula*) is merely a little silique (*siliqua*), as its name implies;—the differences are merely those of size and proportion. Miss Saunders holds that in the silicle the fertile carpel is composed not only of a central part bearing the ovules, but also of a wing-like structure on each side, extending for varying distances in different genera, until it fuses with the margin of the valve carpel (text fig. 9). In other words, what is considered to be the outer, lateral part of the valve carpel in a silique is, under this theory, held to be part—the wing—of the fertile carpel.

The number, origin, and course of the bundles to the fertile carpel in a silique, and to the median, thickened region (the ovule-bearing part) of the semi-solid carpel in a silicle is exactly the same. The authors have shown that the ventral bundles indubitably mark the approximate limits of a carpel. Their number, position, and orientation show clearly that in the solid carpel the carpel margins are uprolled and united; exactly the same anatomy obtains in the rod-like center of the semi-solid carpel, and the ovules are borne in the same manner. Therefore the wings of the semi-solid carpel cannot represent the margins of the carpel.

Miss Saunders' evidence for the existence of this type of carpel rests chiefly upon the secondary venation of the sterile and fertile carpels. The

dorsal bundle of the fertile carpel in this type of fruit gives off branches at intervals which pass across the region of dehiscence of the valves and enter the sterile valve (text fig. 12). Here they approach the region in which are found the terminations of bundles which are branches from the large central bundle of the sterile valve. The region in which the bundles from each carpel meet on the broad face of the fruit is, according to Miss Saunders, the limit between the valve carpel and what she has termed the semi-solid carpel.

If this view is accepted, several features of ovary structure remain to be explained. First, in the silique, as well as in the silicle, bundles, even strong ones, pass from the dorsal bundle of the fertile carpel across the line of dehiscence and enter the tissue of the valve carpel. This can be observed in many genera. Miss Saunders has herself admitted that such conditions exist, though the bundles are said to be few—"in the typical siliqua, in which there are but few vascular connections between valves and replum" If carpel limits are determined by the limits of the lateral veins departing from the midrib of the carpel, all crucifer fruits must have the same morphology, possessing semi-solid and valve carpels, or the semi-solid carpel does not exist in this family.

The lateral branches of the vascular systems of both sets of carpels anastomose at their tips. This is the common condition in syncarpous ovaries, as well as in other fused organs—leaves, petals, etc. So far as bundle tips go there is no sharp organic limit. In fruits generally, the vascular system is that of the ovary; the number and course of the main bundles is not changed, but branches are added, and these may become numerous and strong. These branches may show little respect for carpel limits. The relation of bundles to dehiscence is little also; vascular bundles are broken or split very freely under dehiscence, just as under abscission. Doubtless, as Parkin says (21, p. 197), the primitive dehiscence is along the ventral suture, where no vascular strands need be broken, but there are many cases of transverse and oblique dehiscence wherein bundles are transversely broken, and where carpel lines are surely not followed. The line of dehiscence can not be taken as indicating carpel limit.

The absence in the silicle of contour lines externally delimiting the carpels is considered further evidence in support of the existence of the semi-solid carpel. This absence is explained by the fact that the wings of the semi-solid carpel pass imperceptibly into the edges of the valve carpels. This evidence is negative, at least. The reason why the suture lines are weak or non-existent in silicles lies in the fact that the silicle is a broadened type of crucifer ovary, and the lateral expansion has so flattened the valves and smoothed out the sutures that they are no longer evident to the naked eye. Sections of silicles of many genera however, show the sutures, though of course weakly.

The position of ovules on the semi-solid valve (midway between the

margin and the midrib) is anomalous, as Parkin has shown. Either (1) a migration of the ovules must be assumed, or (2) the wings must be secondary expansions of the solid carpel (which would apparently be closer to Miss Saunders' theory of the nature of the semi-solid type). There is no evidence to support either of these propositions.

One of the chief advantages claimed for the solid carpel theory in the crucifers is that it explains the commissural stigmas. The stigmas are over the midribs of the fertile carpels—the normal position for stigmas. Yet Miss Saunders states that for *Matthiola* the two main stigmatic areas are not commissural, but situated over the midribs of the sterile valves. In other words the stigma of this and other genera is more or less capitate. That the valve carpels may bear independent stigmas is clear from the condition in *Parolinia* (18, p. 352). This genus, with *Anastatica* (1, vol. 3, p. 184) and others make it even more clear that there are two quite separate whorls of carpels in the crucifer pistil, and the outer set are distinctly lower and embrace the inner set.

The primitive condition of the stigma may well be, as Parkin has said, capitate, with all four carpels concerned. With the development of sterility in the outer set, the stigmas of this set may have disappeared, though still remaining as vestigial in some genera. In at least some cases the commissural stigma clearly represents the fused halves of adjacent median stigmas. This is often evident from external morphology, but is demonstrated beyond question by anatomy.

The authors cannot agree with Miss Saunders that there are two sorts of carpel polymorphism in the crucifers; both of her types are in their opinion the same, the variations, including that of lateral venation, being the result of modification of fruit shape. They take much the position of the alternative to her interpretation of the silicle (26) outlined in the footnote on page 141 of her paper: there are four carpels, two solid and two valve, exactly as in the silique, and the lateral venation is a secondary adaptation, the extent and strength of the systems radiating from the midrib bundles being controlled by the shape of the pod and the regions of most rapid ontogenetic growth. The value of secondary nervation to establish carpel structure, is, as Bugnon (2) has recently said, "more than contestable."

On the basis of the interpretation here presented the morphology of the silicle is as straightforward as that of the silique. The dehiscence in each case is normal, falling along the carpel limits.

In respect to the presence of four carpels generally in the crucifers the authors are in agreement with Miss Saunders. That there are 16 carpels in *Brassica* and *Sinapis* and 40–50 in *Rapistrum* they deny on the basis that the longitudinal ribs or bundles in these genera do not represent solid carpels but are merely lateral veins derived from the normal longitudinal veins of the carpels, and have no carpellary significance. Miss Saunders (28) has herself recently recognized this fact and withdrawn her claim.

The carpels are obviously in whorls of two, and there are two whorls generally present, one definitely above the other (just as in the sepals one of the two whorls is markedly above the other). Additional carpels often occur, nearly always in pairs, a solid and a valve. This apparently indicates that extra whorls of two are present and that more than four carpels were characteristic for forms ancestral to the crucifers. In *Conringia orientalis* (L.) Dumort., *Raphanus Raphanistrum* L., and *Cheiranthus Allionii* there is found anatomical evidence of the former existence of these carpels. After the ventral bundles of the solid carpels pass from the receptacular stele there are left strands which pass on into the top of the receptacle and there die out. There can be no other explanation of these bundles but that they are vestigial traces to a third, higher whorl of carpels.

Supernumerary carpels are of frequent occurrence in some genera. Prain (24) in 1898 described and figured various forms of *Brassica* in which the four-valved forms occur with more or less frequency. He also illustrated other types of fruit abnormality in this genus.

ALLIED FAMILIES

The families closely allied to the crucifers have not as yet been investigated by the authors to the extent demanded by their probable significance in the matter of the carpel morphology of the crucifers. The anatomy of several forms in the *Capparidaceae* and *Fumariaceae* has, however, been thoroughly studied. The general structure of the ovary in these forms is closely similar to that of the crucifers, differing chiefly in the absence of the septum. It is usually considered that there are present two carpels, accompanied by a unilocular condition, although some of the *Capparidaceae* may possess more than this number of carpels. As to the nature of the placental region in these groups the authors are again in agreement with Miss Saunders, believing with her that such regions represent consolidated carpels—the “solid” carpels of the *Cruciferae*. Their evidence is, however, again different from that presented by Miss Saunders, being derived from the vascular anatomy of the base of the ovary. The anatomy of this region is identical with that of the corresponding region of a cruciferous ovary. A diagram of the basal region of *Polanisia graveolens* Raf. (text fig. 11) illustrates this condition. Strong inverted bundles face the dorsal bundles of the fertile carpels, the two bundles together making up the vascular supply usual to a closed carpel. From the inverted bundles is derived the vascular supply to the ovules. The three traces to each sterile carpel are normal in position and orientation. *Cleome spinosa* L. has closely similar anatomy.

In the *Fumariaceae* the bundle system of the solid carpels is apparently reduced, for in several forms, species of *Dicentra*, *Corydalis*, and *Fumaria*, the ventral bundles were indistinct or lacking, being evidently fused with the dorsals. However, in the larger flowers of *Dicentra spectabilis* Lam. they are clearly present, and it is probable that the examination of a larger

number of species than the authors have had available will reveal them in other forms. (The species studied, other than *D. spectabilis*, are all small delicate herbs.) The anatomy of the ovary of this larger form shows (text fig. 14) the typical three traces to all four carpels. The ventrals of the solid carpels are only partly inverted. These are, however, undoubted ventrals since they arise in the usual order and position and give rise to the ovule traces. In *D. Cucullaria*, *D. canadensis*, *Corydalis aurea*, *C. sempervirens*, *Adlumia fungosa*, and *Fumaria officinalis* the ventral bundles are apparently lacking and the ovule traces are derived from the "dorsal" bundle. That this bundle is compound seems to be evident from its structure and it is probable that the ventrals have become fused with the dorsal. In these delicate flowers the solid carpel is thus apparently further reduced, and a condition similar to that found in many achenes obtains—the ventrals are fused with the dorsals, and the ovules derive their vascular supply from the one (compound) vein. Among the crucifers only *Arabis* of the many genera studied shows an absence of these fertile carpel ventrals, and this genus is generally recognized as among the most highly specialized of crucifers. In ovary structure at least it is evidently much specialized, the solid carpels being more reduced than in most genera.

The solid carpels of the Capparidaceae and Fumariaceae lack the expanded inner margins which in most crucifers divide the loculus. Whether this septum has disappeared in these families—it exists as a marked ridge in the Capparidaceae—or has never been present in ancestral forms, cannot be said until the Papaveraceae and other forms in these families are thoroughly studied.

Since in both "bi-carpellary" and "multi-carpellary" Capparidaceae the loculus becomes "incompletely divided by placental ingrowths," and since the Fumariaceae have advanced zygomorphic flowers, it seems probable that the septum has disappeared in these families, and that there is present an ovary more specialized than that of the crucifers. Suggestions concerning the interrelations of these families, and their relation to the clearly ancestral papaveraceous stock, cannot be made until thorough studies of the comparative morphology and anatomy of these groups is made.

SUMMARY

1. There are two kinds of carpels in the Cruciferae; a valve, or "sterile" carpel, and a "solid" or "fertile" carpel. These carpels are arranged in two whorls of two carpels each. The outer whorl is made up of two valve-like, open carpels bearing no ovules. The inner whorl consists of two solid or closed ovule-bearing carpels, in which the loculus has disappeared by reduction.

2. The evidence for this rests chiefly upon the vascular structure of the base of the ovary. The solid carpel, regarded by many as the placental region, is found to have the same vascular supply as other carpels. This

consists of a dorsal, or midrib bundle, and two lateral, or ventral bundles. From these lateral bundles is derived the supply to the ovules.

3. The dissepiment is believed to be an expansion of the ventral margin of the folded solid carpels. The evidence for this rests upon the course of the bundles in the base of the solid carpel; the solid carpel has evidently contracted, resulting in the disappearance of the loculus.

4. The peculiar position of the ovule, situated in the loculus of the carpel to which it is not attached, is explained. The ovules, attached to the solid carpel, have pushed out, or evaginated, the dissepiment. They are thus surrounded by a pellicle representing carpellary tissue. In many cases this pellicle, primitively present, has disappeared by reduction, although it persists on the funiculus of the ovule.

5. The authors disagree with Miss Saunders as to the presence of a semi-solid type of carpel in the Cruciferae, and consider that there are but two types of carpels present.

6. Based upon the same type of evidence, the Capparidaceae and Fumariaceae are considered to have solid and valve carpels.

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MINERAL NUTRITION AND CHLOROPHYLL DEVELOPMENT IN SEEDLINGS¹

CARL G. DEUBER

(Received for publication November 25, 1927)

It was observed from class-room experiments that some seedlings, when grown in distilled water, have smaller but darker green leaves than seedlings grown in a so-called complete nutrient solution. In order to account for the apparently peculiar chlorophyll physiology involved, the writer made an analysis of the growth and chlorophyll² conditions of such seedlings.

Wilson soy bean seedlings were used in this study. The seedlings were grown in the usual manner of conducting mineral nutrition experiments with water cultures. Knop's mineral nutrient solution of the following composition was used: $\text{Ca}(\text{NO}_3)_2$, 0.8 g.; KNO_3 , 0.2 g.; KH_2PO_4 , 0.2 g.; MgSO_4 , 0.2 g.; FeSO_4 , 0.0049 g., and distilled water to make 1,000 cc. Distilled water is referred to as a "low plane of mineral nutrition" and Knop's mineral nutrient solution as a "high plane of mineral nutrition." The culture solutions were renewed at the end of a week.

The chlorophyll from 1 to 10 g. samples of fresh cotyledons or fresh leaves was extracted according to the method of Schertz.³

EXPERIMENTAL

The progress of the soy bean seedlings in experiment 21 will be briefly described as typical of the more than twenty experiments performed. The seedlings when 6 days old were transferred to the culture vessels. Distinct greening of the yellow cotyledons of the seedlings occurred within 4 hours after exposure to light. After 2 days, the green color of the tops appeared the same in distilled water and in Knop's solution. The roots of the seedlings in Knop's solution were pure white, turgid, and with 10 to 12 lateral roots, while the roots in distilled water consisted of a straight main root, yellow to brown in color, with only the slightest protuberances to indicate where laterals might form.

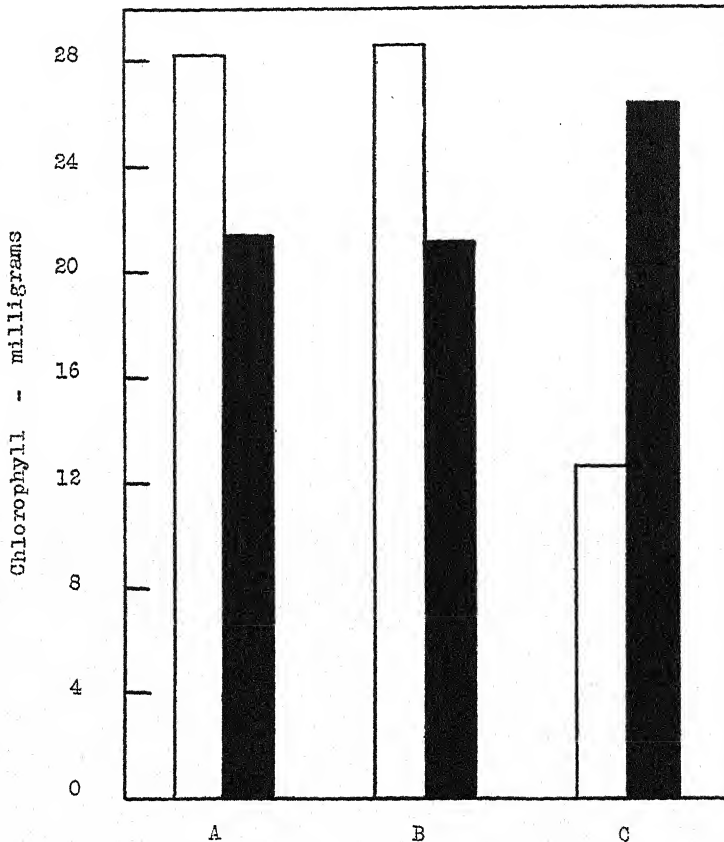
After 4 days, the seedlings in distilled water were quite small compared with those in Knop's solution. Red pigment was evident in the stems of these small seedlings. The cotyledons were smaller but darker green than those of seedlings in Knop's solution. Similarly, the first leaves were

¹ Contribution from the Osborn Botanical Laboratory, Yale University.

² Chlorophyll in this paper refers to combined chlorophyll alpha and beta.

³ Based on a communication from F. M. Schertz, Bureau of Plant Industry, U. S. Department of Agriculture.

smaller and darker green. On the seventh day, the small leaves of seedlings in distilled water showed a yellowing of small areas at the leaf tips and margins. These leaves were not as succulent as those of seedlings grown in Knop's solution.



TEXT FIG. 1. Block diagrams of chlorophyll contents of: A, 10 g. fresh first leaves; B, 10 sq. dm. fresh first leaf area; C, 100 fresh first leaves. The white blocks represent data of seedlings grown in distilled water, the black of those grown in Knop's solution. Data from experiment 2. Soy bean seedlings grown in cultures 15 days (March 22 to April 6, 1926). Seedlings 4 days old when placed in the culture vessels.

By the eleventh day, the seedlings in distilled water appeared to be dying but the non-chlorotic middle and bases of the first leaves retained a darker green color than corresponding but larger leaves of the Knop's solution set. The first compound leaf of these dwarfed seedlings did not unfold, whereas those in the Knop's solution set were about one-half their mature size. Plate XVII shows the appearance of typical seedlings grown with low and high mineral nutrition for 20 days.

The results of experiments 1 and 2 are presented in table 1 and text figure 1, respectively. These experiments were conducted in 1926 and differ from similar experiments in 1927 in that growth and chlorophyll data were taken but once, at the conclusion of the experiments. These results also take into account the area and weight of the first leaves.

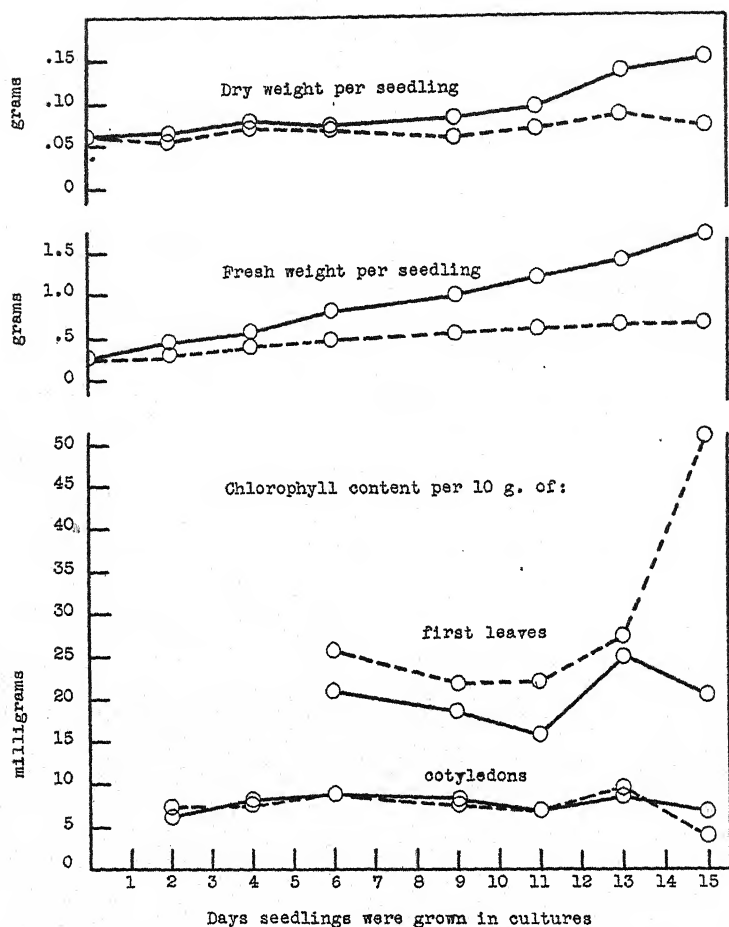
TABLE 1. *Data from Experiment 1. Soy Bean Seedlings Grown in the Cultures 14 Days (June 8 to June 22, 1926); Seedlings 6 Days Old When Placed in the Cultures*

	Culture Media	
	Distilled Water	Knop's Solution
Average fresh wt. per seedling: g.....	0.61	1.07
Average fresh wt. per first leaf: mg.....	36.72	81.02
Average area per first leaf: sq. cm.....	3.91	8.19
Chlorophyll per 10 g. leaves: mg.....	31.29	21.30
Chlorophyll per 10 sq. dm. leaves: mg.....	29.30	21.00
Chlorophyll per 100 first leaves: mg.....	11.40	17.20

In table 2 and text figure 2 growth and chlorophyll data were taken at short intervals during the progress and at the end of the experiments. The object was to determine as nearly as possible the time when the processes controlling chloroplast pigment formation were influenced by the plane of mineral nutrition given the seedlings. With this end in view the chlorophyll conditions existing in the cotyledons as well as in the first leaves were determined.

TABLE 2. *Data from Experiment 22. Soy Bean Seedlings Grown in the Cultures 12 Days (Aug. 1 to Aug. 12, 1927); Seedlings 5 Days Old When Placed in the Cultures*

Days Grown in Cults.	Culture Media							
	Distilled Water				Knop's Solution			
	Wt. per Seedling		Chlorophyll per 10-g. Samples		Wt. per Seedling		Chlorophyll per 10-g. Samples	
	Fresh	Dry	Coty- ledons	First Leaves	Fresh	Dry	Coty- ledons	First Leaves
	g.	g.	mg.	mg.	g.	g.	mg.	mg.
1.....	0.389				0.389			
2.....	.341				.366			
3.....	.459				.471			
4.....	.428		7.97		.620		6.29	
5.....	.469		7.03		.771		7.43	
8.....	.600		13.95	27.41	1.026		6.93	19.84
10.....	.613		11.69	28.02	1.147		10.66	26.37
12.....	.673	0.057	8.06	28.07	1.265	0.079	8.56	20.56



TEXT FIG. 2. Data from experiment 23. Soy bean seedlings grown in cultures 15 days (Sept. 3 to Sept. 18, 1927). Seedlings 5 days old when placed in the culture vessels. Continuous lines show data of seedlings grown in Knop's solution; broken lines, data of seedlings grown in distilled water.

In the graphs of chlorophyll concentration of first leaves on the 15th day, the very high value of chlorophyll concentration of the leaves of seedlings grown in distilled water may require explanation. These leaves were very small (it would have required 588 of them to make a 10-g. fresh leaf sample). They were yellowed around the margins and up the tip with only the base and middle of the blade dark green. Also, these leaves were very dry to the touch and portions of the leaf margins were dry and curled. Leaves from seedlings grown in Knop's solution were relatively large (requiring 72 to make a 10-g. fresh leaf sample), of a normal green color, and quite succulent.

DISCUSSION

Throughout this investigation it has been found that wherever dwarfing of the soy bean seedlings occurred due to growing them in distilled water the chlorophyll content per 10 g. of fresh leaves was higher than in com-

parable leaves of seedlings grown in Knop's solution. The total amount of chlorophyll per leaf, or per 100 leaves, as the results are presented in table 1 and in text figure 1, was less in the leaves of the seedlings grown with a low plane of mineral nutrition than in the leaves of seedlings grown in Knop's solution. An example of these differences may be taken from table 1. The chlorophyll content per 10 g. of fresh leaves of seedlings grown in distilled water was 31.29 mg., while in similar leaves, but from seedlings grown in Knop's solution, the chlorophyll content was 21.30 mg. But, per leaf, the chlorophyll content was 0.11 mg. from seedlings grown in distilled water and 0.17 mg. from seedlings grown in Knop's solution. Results of this nature throughout the investigation indicate that leaf for leaf in the two types of seedlings, size of leaf, either fresh weight or area, was reduced more than chlorophyll content was reduced by the low plane of mineral nutrition. A similar relation exists between fresh or dry weights of entire seedlings and the chlorophyll contents of the more important chlorophyll containing organs—the cotyledons and first leaves.

Chlorophyll content, quantitatively at least, was not the limiting factor in the dwarfing of the soy bean seedlings grown in distilled water; in fact, there was always *more* chlorophyll produced per gram or per square centimeter of first leaves in the low plane of mineral nutrition than in those grown with a high plane of mineral nutrition. This latter fact accounts for the darker green appearance of the leaves of the dwarfed plants.

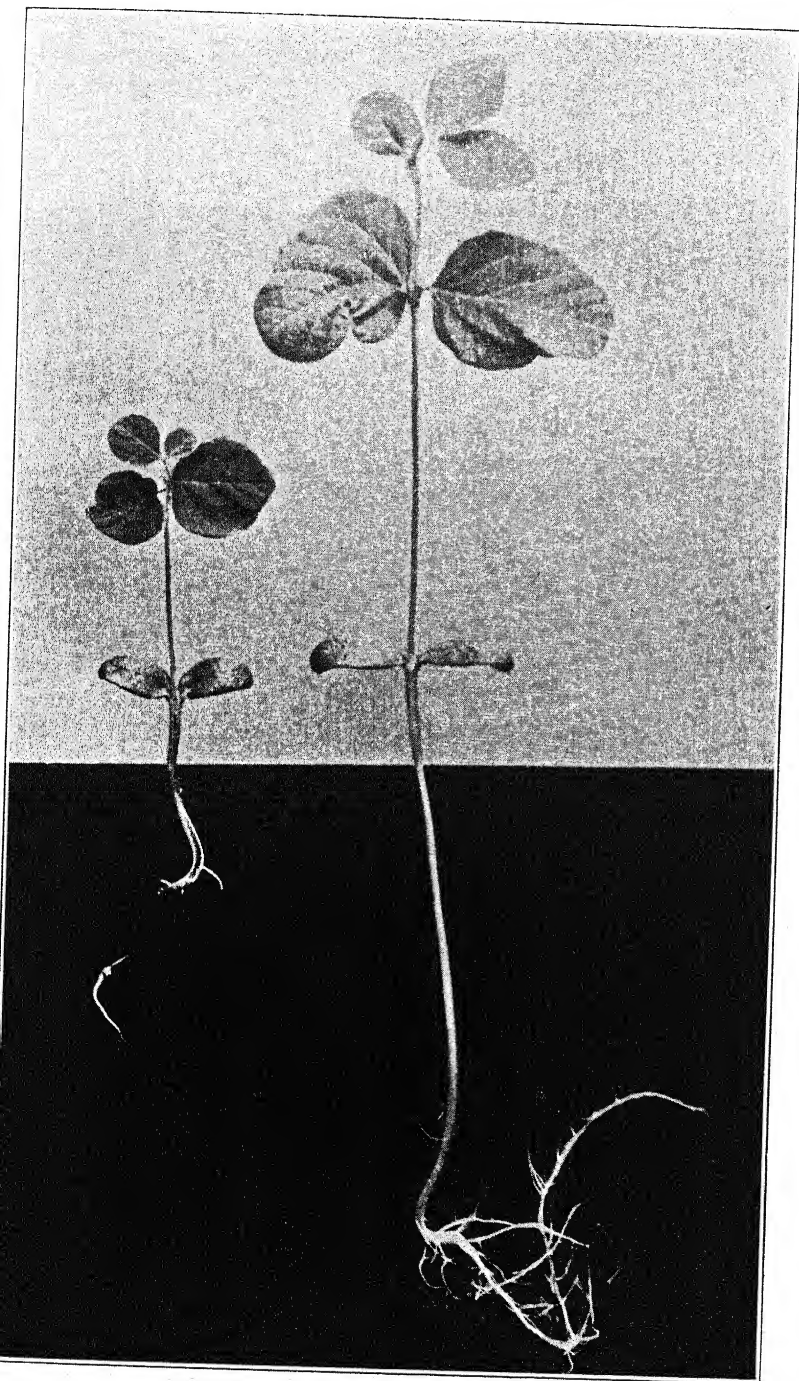
The time required for detectable differences in chlorophyll formation to become apparent, as a result of the plane of mineral nutrition to which the seedlings have been subjected, appears to be very brief. In table 2, differences in the concentration of chlorophyll in the cotyledons were apparent 4 days after the seedlings were placed in the cultures and very significant differences were found between the concentration of chlorophyll in the first leaves when first extracted, on the 8th day in the cultures. Cotyledons appear to be less influenced in the formation of chlorophyll than the first leaves as illustrated in text figure 2. Probably the stored mineral supply in the cotyledons accounts for their greater stability toward external planes of mineral nutrition. In the experiment just referred to, the first leaves showed a marked difference in chlorophyll concentration by the 6th day in the low and high planes of mineral nutrition. Earlier it has been pointed out that distinct stunting of root growth is apparent within 2 days in distilled water. It appears as if disturbances to a satisfactory plane of external nutrition are first reflected in a stunting of root growth, then in top growth, followed by a lessened stunting in the formation of chlorophyll. This results in a relatively high concentration of chlorophyll in the small cotyledons and first leaves of dwarfed soy bean seedlings over a limited growth period.

CONCLUSION

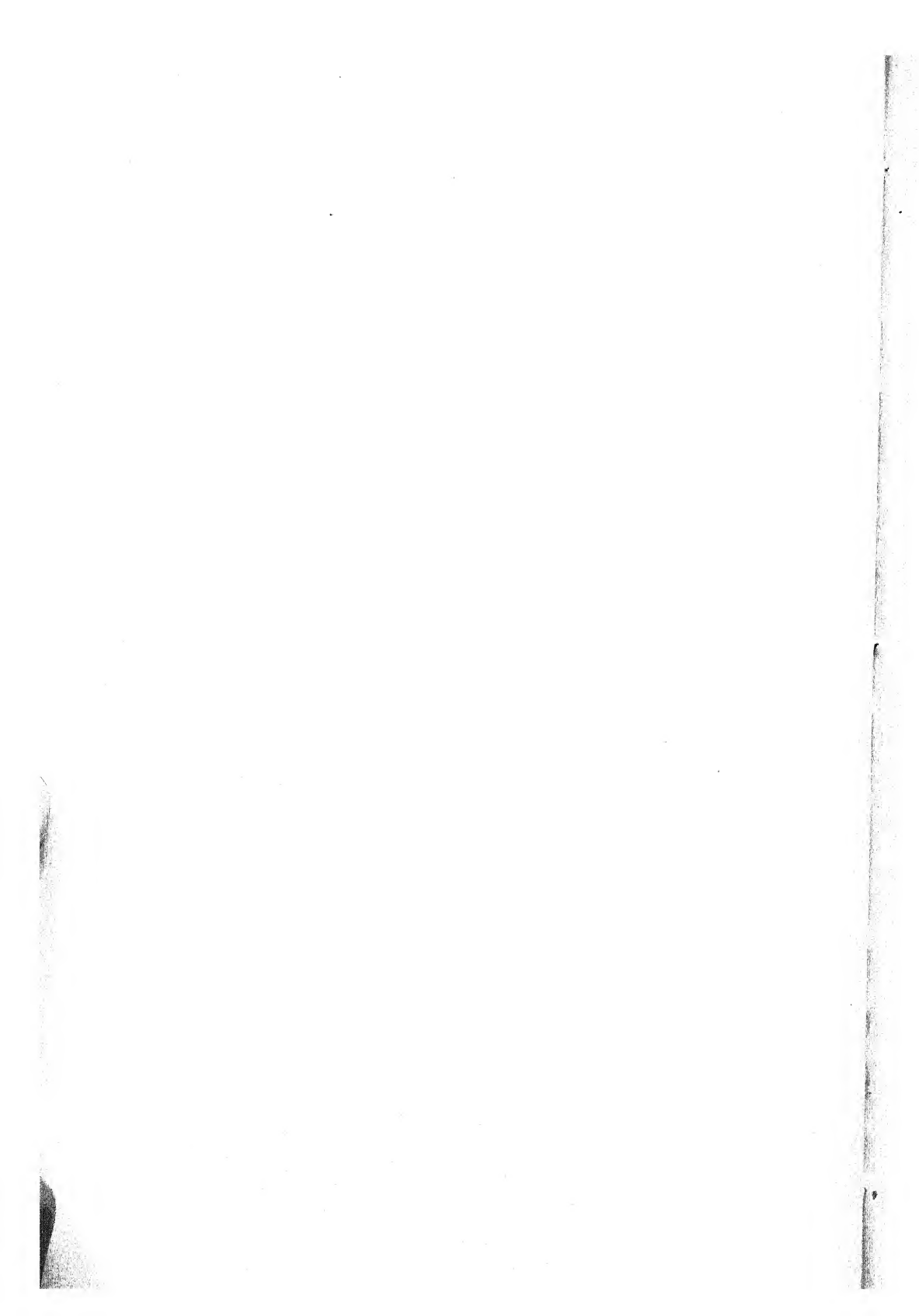
The dwarfing of soy bean seedlings grown in distilled water is accompanied by a higher concentration of chlorophyll particularly in the first leaves and to a lesser extent in the cotyledons. This condition accounts for the tops of such dwarfed seedlings appearing dark green as compared with the normal green color of seedlings better nourished. This condition probably results from the fact that the growth processes in seedlings given a low plane of mineral nutrition are impaired to a greater extent than the processes controlling chlorophyll formation.

EXPLANATION OF PLATE XVII

The smaller soy bean seedling was grown in distilled water, the larger one in Knop's solution. Seed sown December 1, 1927; seedlings transferred to culture vessels December 7, and photographs taken December 27, 1927. The development of these seedlings was considerably slower than that of seedlings grown in the spring and summer.



DEUBER: CHLOROPHYLL IN SEEDLINGS



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THE CHLOROPLASTS OF *ISOETES MELANOPODA*

ROBERTA MOHLING MA

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Marquette's studies (5) on the plastid-like aggregations of starch grains in the cells of young leaves of *Isoetes* and in the spore mother cells of *Marsilia* and *Equisetum* lead him to doubt their identity as plastids; he calls them, instead, "polar structures." Referring to the above fact, he says:

The suspicion arises that possibly the structures found in *Selaginella* and in the spore mother cells of *Anthoceros* are not chloroplasts at all but that the chloroplasts lie inside of these structures, close about the starch grains. This, however, can only be determined by further investigation. There are undoubtedly points of resemblance between the polar structures of *Isoetes* and the bodies described as chromatophores in *Anthoceros*.

Marquette (5) found in cells of very young leaves of *Isoetes lacustris* that abundant starch grains are present and collected in masses. During the resting stage there is present only one aggregation of starch grains, usually closely pressed against the nucleus. In the later stages of mitosis there are regularly two masses present at the poles of the cell. They are located finally near polar depressions in the nuclei of the young daughter cells. These are the starch masses which he designates as "polar structures." They are made up of masses of starch grains lying in clear spaces which are surrounded by a more or less distinct boundary. "Frequently it appears as though a well-defined membrane surrounded the mass of starch grains, a membrane of varying thickness." Among the starch grains are small red-staining granules. These granules cannot be distinguished with certainty from minute starch grains in preparations with triple stain, but with iron haematoxylin the starch grains appear almost colorless and the granules a deep black.

Haberlandt (2, 3) has described the structures in *Selaginella* to which Marquette has referred. They are green, thimble-shaped masses of chlorophyll-bearing protoplasm, and are chloroplasts just as clearly as the single green structures in vegetative cells of *Anthoceros*. Marquette has not expressed doubt as to the identity of the vegetative chloroplasts of the latter. Both plastids contain groups of starch grains possibly similar to the "polar structures" of *Isoetes*.

Davis (1) was unable to demonstrate plastids in young cells of the

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sporogenous layer of *Anthoceros* and therefore did not connect the plastids of the spore mother cells with those of the vegetative cells. There seems but little doubt, however, that the structures described as plastids from the spore mother cells of *Anthoceros* by Davis, and much earlier by Strasburger (6), are true plastids.

McAllister. (4) has shown that the starch-bearing areas in the spore mother cells of *Anthoceros* and *Notothylas* are plastids. The history of the plastids is followed from the time of the differentiation of the sporogenous cell to the formation of the four starch-bearing regions in the spore mother cell. The plastids of the young spore mother cell are small and not unlike the plastids of sporogenous and elater cells. Later, as the mother cell enlarges, the plastids enlarge and become vacuolate. Numerous starch grains then develop in them. The connection of the plastids of the sporogenous area with chloroplasts of the external vegetative layer is easily shown. These plastids of the spore mother cells of *Anthoceros* and *Notothylas* are very similar to the plastids in the leaves of *Isoetes*. The red granules lying in a vacuolate plastid among starch grains in the spore mother cells of *Anthoceros* and *Notothylas* strongly suggest the red granules of *Isoetes* to which Marquette has called attention and of which the writer has made further study.

MATERIAL AND METHODS

The material used for this study of *Isoetes melanopoda* Gay & Dur. was obtained from small ponds in the vicinity of Bastrop, Texas. In late spring the leaves die down to the stem, and during the summer and autumn months the plant lies dormant. The first material was collected January 6, very soon after growth had begun. This material showed, along with the old plants, numerous sporelings less than a centimeter in length. Other collections were made January 31 and February 12. The youngest material was by far the most favorable for this study.

Both young leaves and sporelings were killed in Flemming's weak solution and Merkel's fluid. The plants killed by the latter fixative seemed to give the better results. For this study, most of the slides were stained with Flemming's triple stain, although Heidenhain's iron haematoxylin stain was also used. Flemming's stain gives a red color to certain granules present in the plastid, a violet or blue stain to starch grains, and an orange-yellow stain to cytoplasm. The granules, which stain red with Flemming's stain, are stained black in the iron haematoxylin stain. Iron haematoxylin has no effect upon starch grains.

THE IDENTITY OF THE PLASTIDS

In cells near the bases of very young leaves, the aggregations of starch grains which Marquette called "polar structures" are clearly seen. In *Isoetes melanopoda* these structures appear as rounded or elongated areas which, if not vacuolate, are very poor in protoplasm. Very little stainable

material seems to be present in these areas except certain red-staining granules and numerous small, blue-staining starch grains. The areas are surrounded by a definite, narrow, orange-staining boundary which is clearly distinct from the surrounding cytoplasm.

The present paper shows that by a process of division these "polar structures" give rise to eight or more structures which are, without doubt, plastids. In the following account of their development, the term plastid will be used instead of "polar structure."

At the bases of young leaves, all cells whose nuclei are not in some of the phases of division have but a single large plastid (Pl. XVIII, figs. 1, 2, 3). This plastid lies in close contact with the nucleus, but in no definite position in relation to the poles of the nucleus or cells. Since the nuclear figure also coincides with the long axis of the cell the poles of the cell can be said to be at the upper and lower ends. In some cases the approach of cell division is coincident with the first division of the plastid (figs. 3, 6, 7). In other cases two plastids may be seen in cells, the nuclei of which show no evidence of division (fig. 4). After the plastid has completely divided, the halves withdraw from each other until they lie at opposite ends of the cell, with the nucleus between them (fig. 5). At metaphase and later the daughter plastids are found at the opposite poles of the spindle (figs. 6, 7). It will be seen from the vacuolate condition of the cell at this stage that they could not occupy any other position. The polar region, only, affords sufficient room for the large plastid.

In the mature cells of the upper part of the young leaf, where nuclear division seems to have entirely ceased, the plastids continue to divide. In this part of the leaf various numbers of plastids are to be seen in the cells, but apparently never a single plastid only (figs. 8, 9, 10). Most cells contain two plastids (fig. 5). The size, structure, and contents of these plastids are the same as of those in cells at the base of the young leaf where nuclear and cell division is still active. In no recognizable way do they differ from those which are to be observed in dividing cells.

Even though there be only two plastids in a cell, these have no definite and fixed position with respect to the poles of the cell. In cells in which the nuclei are actually dividing, the plastids seem to be regularly at the poles of the spindle (figs. 6, 7). At other times, *i.e.* when the cells are not dividing, the plastids are somewhere near the nucleus. These plastids are located usually in regions where protoplasm is most abundant (figs. 5, 8). From the sections studied, it is seen that the plastids have no tendency to occupy a polar position except during the nuclear division (figs. 6, 7).

In older cells, the two plastids are to be found dividing to form four (figs. 8, 9). These four plastids seem to be similar to the parent plastids, except as to their shape. They are usually oblong in shape, though more rounded than the parent plastids. The starch content tends to be greater in these older cells. This increase is probably due to better exposure to the light and to smaller demand for food material by the mature cell.

The position of the four plastids, as in the case of the two plastids, seems to be determined by the position of the larger masses of cytoplasm and by the location of the vacuoles (figs. 8, 9). The plastids are always quite close to the nucleus but are arranged with no definite relation to the axis of the cell.

In still older cells, about a centimeter above the base of the leaf, a part or all of the four plastids seem to have divided. Cells with six or seven plastids were found to be common (fig. 10). The variation in number is probably due to the fact that the four plastids rarely divide simultaneously. There is no essential difference between these plastids and those found in younger cells. Their shape seems to be more or less rounded and smaller than the parent plastids.

The development of cells with numerous plastids from the cells with a single one is clearly shown in the above observations. If those plastids of the dividing cells have a definite relation to the nucleus during division and are truly "polar structures," as Marquette believed them to be, they lose that polar relation in older cells and become true plastids. Even if they can be shown to possess the polar relation ascribed to them, there is no reason to call them by any other name than plastids. It seems more probable, however, that the plastids have only accidental relation to the poles of the nucleus. The position of these large plastids in the cell is circumscribed and determined by the location of the larger masses of cytoplasm. Since the position of the spindle poles of the nuclear figure is determined by the same cause, the spindle poles and the plastids lie adjacent.

THE NATURE OF RED-STAINING GRANULES IN THE PLASTID

Mention has already been made of Marquette's reference to red-staining granules lying among the starch grains of the "polar structures" in *Isoetes lacustris*. Such bodies have also been mentioned incidentally by the author as occurring in plastids of *I. melanopoda*.

The similarity of these red-staining granules to the red-staining bodies to be seen in the plastids of *Anthoceros* and *Notothylas* (4) is of special interest. In these Hepaticae, the red bodies lie in vacuolate plastids scattered among starch grains many of which are not unlike the red granules in shape and size. In the cells of the deeper lying vegetative tissue of the sporophytes of these plants are found characteristic, red-staining "pyrenoid bodies" scattered through the plastid. "Even the cells of the growing intercalary area show scattered pyrenoid bodies mixed with starch grains of the same general shape. The shape of the starch grains of the vegetative cells of the sporophyte is the same as in the gametophyte."

McAllister has paid special attention to the pyrenoid bodies in the developing spores of *Anthoceros* and *Notothylas*, the plastids of which have much in common with the large, single plastids of *Isoetes*. In the young cells of the sporogenous layer, the chloroplasts are similar in appearance to

those of other layers of the same age. As the spore mother cells become definitely organized the plastid divides twice, forming the four plastids of the four nascent spores. The protoplasm outside of the plastid and also of the plastid itself becomes vacuolated. In the vacuole-like region of the plastid there appear numerous scattered safranin-staining bodies which are usually rounded in form.

In some plastids small violet-staining bodies are to be seen which are scarcely larger than the red-staining pyrenoid bodies and differing from them visually only as to color and size. "A tendency on the part of the young starch grains to be arranged in linear rows is often to be observed, which fact also suggests their origin from rows of the red-staining bodies. With the development of starch grains in the vacuolate area of the plastid, there is an appreciable decrease in number of pyrenoid bodies." These red granules lying in a vacuolate plastid among starch grains, in the spore mother cells of *Anthoceros* and *Notothylas*, show a striking similarity to the "small granules taking red stain scattered among the starch grains" in the cells of the leaves of *Isoetes* to which Marquette has referred.

In the plastids of *Isoetes melanopoda* there are violet-staining starch grains and safranin-staining granules lying scattered through the interior of the plastid (figs. 1, 3, 4, 5, 6, 7, 8, 10). The scant protoplasmic content of the plastid often appears in the form of delicate strands. At other times it is scattered in irregular masses. Some plastids seem even to lack any traces of stainable material except the starch grains and red-staining granules.

In the growing cells both the starch grains and the red-staining granules are very minute, measuring not more than 0.5 micron in diameter. The staining reaction of these two classes of bodies is usually clear-cut and distinct, but frequently in the same preparation plastids may be observed in which neither the starch grains nor the red-staining bodies take a brilliant, distinct stain. In these latter plastids it is not possible to distinguish with certainty between the two classes of bodies. It seems improbable that this indefiniteness of staining reaction is due to variation in the staining technique since neighboring cells in the same preparation may show a variation in the reaction to the violet and red stain. It seems more probable that two such unlike cells represent two distinct physiological conditions in which one cell may be forming starch actively while the other may show starch undergoing hydrolysis.

During the division of the plastid, both the red granules and the starch grains seem to decrease in number (fig. 4) due to their distribution between the daughter plastids, but after division the number of starch grains and of red granules has a tendency to increase (fig. 5). Because of the minuteness of the red granules, it is difficult to determine whether they increase by the process of fission or whether the new granules arise *de novo*. Likewise, it is difficult to determine whether the new starch grains are trans-

formed red granules or whether they come from some other source. The striking similarity of red granules and starch grains in point of shape, size, and position in the plastids makes the former method seem very probable.

The plastids found in the mature cells contain fewer red granules and starch grains than those in growing cells (figs. 8, 10). This decrease may be caused by the continual division of the plastids. In the outer leaves of some older plants, however, no red granules can be found, but the starch grains are abundant and very much larger (fig. 9) than in similar cells in young leaves.

In view of the similarity of the starch grains and the red granules in the plastids of *Isoetes* as to size, shape, and location, it seems probable that the red granules are "primordia" of the starch grains, becoming transformed into rudimentary starch grains. The pyrenoid bodies in the plastids of the cells of *Anthoceros* and *Notothylas* (4) undergo such a change. These latter bodies have the same staining reaction as the red granules, and the starch grains stain blue with triple combination. The pyrenoid bodies give rise to rudimentary starch grains, which increase in size to form mature grains.

DISCUSSION

According to the above observations, it is evident that the chloroplasts of *Isoetes melanopoda* lie in various positions with respect to the poles of the cell, depending upon the age of the young leaf and also upon the physiological activity of the cells. In the cells where photosynthesis is active, from one to eight plastids have been observed. The number, however, probably far exceeds eight in fully developed leaves. In these plastids there are numerous blue-staining starch grains and red-staining granules lying mixed together. The interior of the plastid appears in some cases to contain scattered protoplasm; in other cases it seems to be a vacuolate area which is surrounded by a narrow zone of compact protoplasm. These plastids do not differ conspicuously from the "polar structures" which Marquette (5) has described for *Isoetes lacustris*. This study shows that when there are but two plastids in a cell they occupy approximately a polar position; but when a cell contains four or eight plastids, it is impossible for them to be arranged in a polar position with respect to the nucleus.

In the "polar structures" of *Isoetes lacustris*, described by Marquette (5), the blue-staining starch grains obtained by the use of triple stain are even more conspicuous than those in the plastids of *I. melanopoda*. He describes small red granules scattered between the starch grains as scarcely to be distinguished from minute starch grains in his preparations. In some preparations of *I. melanopoda*, the writer has found the red granules easily distinguishable from the minute starch grains. In other preparations the contrast between the two kinds of bodies is less clear. It seems unlikely that this difference is due to imperfect staining. It is probably due to the

physiological condition of the plastids. It is possible that these less distinct bodies may be transitional stages from the red-staining granules to the blue-staining starch grains.

These minute granules are similar in staining reaction to the "pyrenoid bodies" of the spore mother cells of *Anthoceros* and *Notothylas*, as described by McAllister (4). The chemical nature of the red granules in *Isoetes* has not been determined, but from the similarity of their staining reaction with standard stains to that of pyrenoids, it seems probable that they are similar chemically. The pyrenoid bodies in the mature spore mother cells of *Anthoceros* and *Notothylas* are rounded and scattered among the blue-staining starch grains and lie in a vacuolate plastid. They are comparable in their relations to one another to the red granules and the violet starch grains in the vacuolate plastid of *Isoetes melanopoda*. McAllister has advanced proof which leads him to conclude that the pyrenoid bodies of *Anthoceros* and *Notothylas* give rise to starch grains. The transitional pyrenoid bodies stain neither blue nor red by means of Flemming's triple stain, but a shade intermediate between the two colors. In *Isoetes*, the evidence is not sufficient to lead to the conclusion that the red-staining granules become transformed into starch grains. Nevertheless, from their similarity to the pyrenoid bodies of *Anthoceros* and *Notothylas* it seems probable that they do undergo such a transformation.

In cells of the leaves of *Isoetes* fully exposed to light, the number of plastids is probably as high as thirty in each cell. Plastid size is correspondingly smaller. The writer has not had opportunity to make careful study of these tissues. From the close similarity in general structure of the plastids in the young tissues, whether the cell contains one or eight plastids, it seems reasonable to expect that in cells with more than eight this similarity in structure should still be found, and that the plastids should show the same red-staining pyrenoid-like bodies as are visible in the younger cells.

The writer wishes to express her appreciation to Dr. F. McAllister, who suggested the problem and gave helpful advice and encouragement.

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EXPLANATION OF PLATE XVIII

The figures were drawn with the aid of the camera lucida. All figures are taken from the cells of the young leaves of *Isoetes melanopoda* Gay & Dur. and are magnified about 1,500 diameters. Red-stained granules are shown in dense black, and starch grains in gray.

FIG. 1. Resting cell. The plastid containing minute starch grains and red granules lies at one side of the nucleus.

FIG. 2. Two cells at beginning of prophase. The nuclei lie close to the cell wall; the plastids contain red granules only, and very scanty protoplasm in plastids.

FIG. 3. An unusually large plastid, evidently just before division.

FIG. 4. Division of plastid.

FIG. 5. Plastid division completed. The daughter plastids are located at the opposite sides of the nucleus.

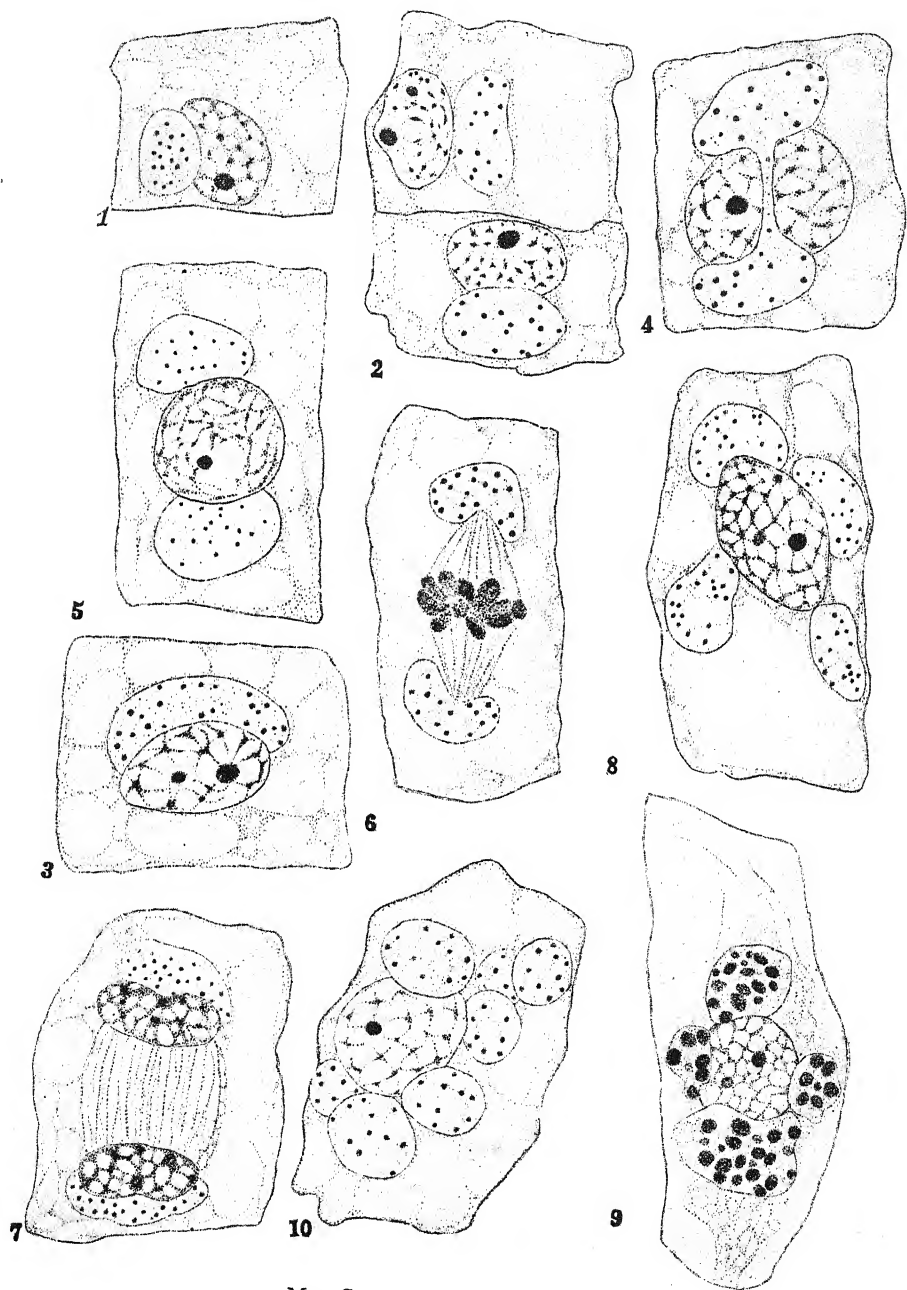
FIG. 6. Metaphase stage of cell. The two plastids are situated at the poles of the spindle.

FIG. 7. Reconstruction of daughter nuclei. These plastids contain almost equal amounts of starch grains and red granules.

FIG. 8. Cell of upper part of the leaf. Four plastids surround the nucleus.

FIG. 9. Cell as in the preceding figure. An abundance of mature starch grains is shown.

FIG. 10. Cell of the uppermost part of the young leaf. Seven plastids are seen around the nucleus, the cell greatly vacuolated.



MA: CHLOROPLASTS OF ISOETES

A STUDY OF THE CATALASE OF THE FRUITS OF PEAR VARIETIES

E. L. OVERHOLSER

(Received for publication December 23, 1927)

This study of the behavior of catalase in the pear was made in an attempt to shed light on the ripening and keeping quality of fruit at both room and storage temperatures.

Not all workers are agreed as to the importance that can be attached to catalase in plant tissues. Many, however, as indicated by a review of the more recent literature, believe that catalase is an enzyme which plays a significant part in plant metabolism.

EXPERIMENTAL METHODS

Hausler (1918) states that since an enzyme preparation is a mixture of compounds, which are partly of a similar nature, it is scarcely possible to determine which constituent is the source of the enzymic action. He therefore believes the nature of enzymes can be explained and can best be studied by experiments on their activity rather than by present methods of purification or partial isolation and chemical investigation of the preparation.

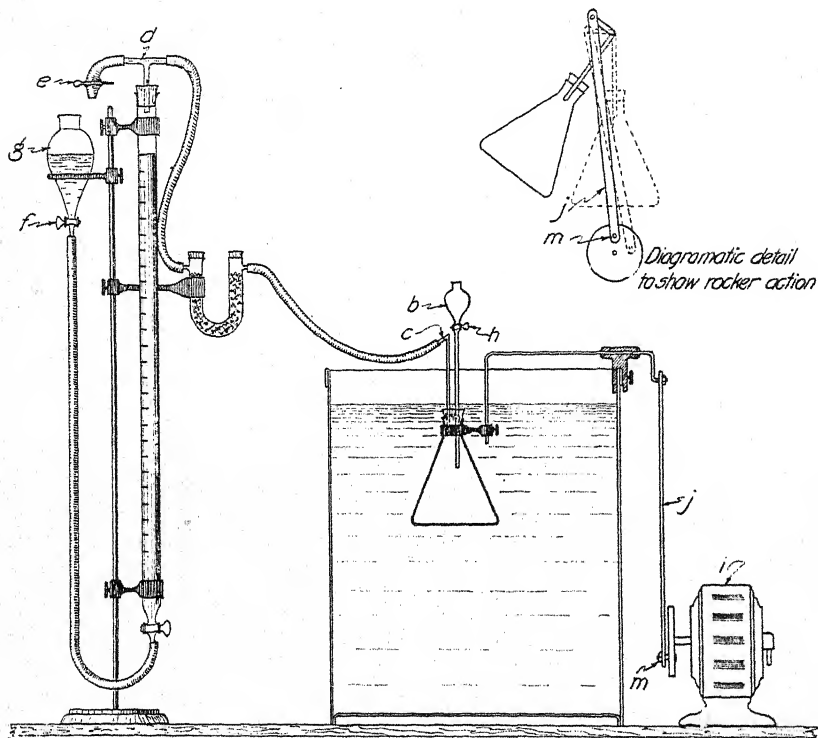
In the conduct of the present work it has been assumed that the view of Hausler is essentially correct. Hence, the enzyme as present in the ground pear tissue was studied without attempting to purify it. It is, however, recognized that the enzyme, which is thus studied by its activity only, may be influenced by the injury and maceration of the cells incident to grinding and may therefore respond differently from the way it does in normal tissues. Nevertheless, from the point of view of the work reported here, this latter method seemed to be the more desirable.

Method Used in the Determination of Catalase Activity

In devising a method for the determination of catalase activity of pears there appeared to be several possible sources of error among which might be included (a) variation in degree of fineness of grinding; (b) rapidity of neutralization of the tissue, and the question as to whether neutralization was advisable; (c) variation in the quantities of the pulp and reagents; (d) order in which the reagents were added and period of time elapsing after the material was prepared before the determination was made; (e) temperature at which the determination was made; (f) rapidity and uniformity of agitation in the reaction chamber; and (g) conditions under which the pears had been previously kept.

The possible causes of experimental error were eliminated or reduced to a minimum by adopting a standard method of procedure, which was followed as closely as possible with each determination.

The method employed was a modification of those used by Appleman (1915), Crocker and Harrington (1918), and Heinicke (1923). The details of the apparatus are given in text figure 1. The modifications were based



TEXT FIG. 1. Diagrammatic details of the apparatus employed in making catalase determinations.

upon experiments by the writer. The reaction chamber consisted of a one-liter heavy Pyrex Erlenmeyer flask, fitted with a two-holed no. 8 rubber stopper. Through one hole was inserted a glass tubing outlet (*c*) attached to rubber tubing connected to one end of a glass T-tube (*d*). One stem of the T-tube was inserted into the top of a 100-cc. glass burette, and the remaining stem was closed by rubber tubing and a pinchcock (*e*). Between the flask and the burette was placed a U-tube containing soda-lime for CO_2 absorption. The hydrogen peroxid was supplied to the reaction chamber by means of a 100-cc. separatory funnel (*b*) with a ground glass stopcock inserted through the second hole of the no. 8 rubber stopper.

The hydrogen peroxid used was kept at a temperature of 2°C . so as to

lessen the possible loss of strength through decomposition. The strength was determined by use of the permanganate method and a 3-percent hydrogen peroxid by weight was used. When the tissue employed had been neutralized with calcium carbonate, the hydrogen peroxid was also neutralized. When, however, the tissue had not been neutralized, the hydrogen peroxid was used without neutralization.

The reaction was carried on at a temperature of 15° C. This was obtained by affixing the flasks to the apparatus shown in text figure 1 so that they were immersed in a water-bath tank (*a*) which measured 76 by 84 cm. As a result of the relatively large volume of water and the addition of cracked ice there was little fluctuation in temperature.

The samples consisted of 100 grams of pulp of pears which with the exception of the stems and the tissue within the primary vascular bundles had been finely ground through a mill, and 100 cc. of distilled water. The equivalent of 1 to 15 fruits was required for a sample, depending upon the size of the variety and the stage of development. One duplicate set each, with and without the addition of an excess of calcium carbonate, was employed for each determination. About 15 grams of calcium carbonate was used per sample.

One of the possible sources of error most difficult to overcome, when calcium carbonate was added, was to neutralize the tissues as macerated before any injury to the catalase might occur from the liberated fruit acids. No consistent difference was found between the catalase activity of a tissue when calcium carbonate was added with the tissue as it was ground and when added after the pulp had been ground and the samples weighed. This probably meant either that little injury to the catalase resulted from contact with the acids because of the relatively high pH value of the pears, or that, notwithstanding the precautions taken, neutralization was not effected before injury had resulted.

The chief reason advanced for the neutralization of the pulp is that only by making conditions favorable for the maximum activity can the amount of catalase be determined. With certain kinds of plant tissues having low pH values it may be important or even necessary to effect neutralization before consistent catalase activity may be demonstrated, as found by Heinicke (1923, 1924). The data obtained, however, indicated that catalase can be measured more definitely by its activity and that nothing can be gained by transposing this activity into a measure of the amount of the enzym present. In most cases where workers have referred to the amount of catalase they have no doubt used this expression as synonymous with catalase activity rather than as an actual measure of its amount.

It thus appears to be of interest to study the catalase from the point of view of its activity instead of its quantity. This being true, it seems illogical to assume that a measure of its potential activity *in situ* is more accurately obtained through modification of the pH value by the addition

of excess calcium carbonate than by making the determination with the pH of the pulp and juice without such artificial modification. Hence, most of the data presented are those obtained without the addition of excess calcium carbonate. In every case, however, tests were made with the addition of calcium carbonate as well as without. The same relative differences between samples was exhibited in either case, although better agreement between duplicate samples was obtained when calcium carbonate was not added. The addition of calcium carbonate, however, always gave greater catalase activity and this increase tended to be greater when the calcium carbonate was added as the tissue was ground.

Heinicke (1924) found that the form of calcium carbonate used is important in that the chemically pure calcium carbonate does not effectively neutralize the macerated tissue. In some unpublished work Heinicke finds that the C. P. calcium carbonate gives no advantage over non-neutralized materials, but that the activity is often increased markedly by precipitated finely divided chalk.

In making a determination the flask and contents were brought to constant temperature; connections tightly made except at the pinchcock (*e*); the water leveled at zero in the burette by means of the leveling bulb (*g*); 25 cc. of the hydrogen peroxid were supplied to the flask through the stopcock (*h*), which was then closed, together with the pinchcock (*e*), making the system air-tight. The motor (*i*) was then immediately started, and the flask and contents were continuously and uniformly agitated by means of the pivoted lever (*j*) geared to the motor at *m*. The back-and-forth movements of the flask thus resulting from one extreme position to the other was 5.5 cm., and the number of such oscillations per minute was 30. The leveling bulb (*g*) was slowly lowered as the gas displaced the water in the burette so that each reading was thus corrected to atmospheric pressure. The amount of water displaced in the burette was taken as a measure of the relative catalase activity. Readings were thus made directly in cubic centimeters at half-minute intervals during six minutes.

Temperature at Which the Test was Made

Vicar pears,¹ harvested August 23, 1924, and stored at 0° C., were removed on February 20 and samples prepared for catalase determinations. The reactions were conducted at temperatures of 5°, 15°, 35°, and 50° C. Blanks were run by using 200 cc. of distilled water instead of the 100 grams of pulp and 100 cc. of water. Data obtained from the average of four determinations indicated a reduction in the catalase activity of pear pulp at a temperature of 5° C., but a marked increase at 15° C. The actual catalase activity of the tissue decreased as higher temperatures were employed. The activity evidenced by the blanks at temperatures of 5°

¹ Most of the fruit employed was obtained from the California Nursery Company at Niles, California.

and 15° C. was negligible. At higher temperatures, however, the splitting up of hydrogen peroxid in the blanks was appreciable, and at temperatures of 35° and 50° C. quite marked. The evidence indicated, therefore, that catalase activity of the pear tissue could be measured most accurately at 15° C.

Variation in Quantities of Materials

Morgulis (1921) stated that the use of large quantities of hydrogen peroxid (equivalent to 500 or 600 cc. of oxygen) is objectionable because the depressing effect is great unless large amounts of the catalase preparations are employed. According to Morgulis, while the oxygen formation is a linear function of the quantity of catalase, this rule does not hold true when either the catalase or the hydrogen peroxid is in great excess.

The tests were made with Angouleme pears removed from the 0° C. storage room in February. The normal treatment was 100 grams of the ground pear pulp, 100 cc. of distilled water, and 25 cc. of 3-percent hydrogen peroxid. Only one of the materials was varied in amount at a time.

The cubic centimeters of oxygen evolved in a given period of time were almost directly proportional to the quantities of hydrogen peroxid used. As the quantity of tissues used was increased the amount of oxygen evolved in a given period of time was also increased even though the amount of hydrogen peroxid added was not varied. The relative increase, however, was not as great as when the amount of hydrogen peroxid was increased. The addition of increasing amounts of water reduced the number of cubic centimeters of oxygen evolved.

It was believed that with 25 cc. of hydrogen peroxid sufficient was available so that hydrogen peroxid would not be the limiting factor. Since, however, doubling this amount of hydrogen peroxid nearly doubled the amount of oxygen evolved, the 25 cc. was not sufficiently large to result in a depressing effect.

The 300 grams of pulp and juice alone absorbed slight amounts of oxygen, possibly as a result of the tannin-like substance present in the pulp (Overholser and Cruess, 1923).

There was no absorption or evolution of gas with the 200 cc. of water alone.

Effect of Boiling the Tissue

Sohngen and Smith (1924) found that the catalase of yeast was destroyed by a temperature of 65° C.

Onondaga pears harvested in the fall of 1924 were utilized in May 1925 from the 0° C. storage room. The procedure was identical with the standard method except that after grinding, weighing, and the addition of the distilled water, samples were boiled for fifteen and thirty minutes, brought to the original volume by the further addition of distilled water, and cooled to the temperature of the bath (15° C.) before the addition of the hydrogen peroxid and CaCO₃. One set of determinations were made as soon as

cooling and constant temperature had been effected and another set after standing for twenty-four hours.

It is recognized that there is doubt as to the nature of the catalase. This question as to whether it may be a true enzyme is pertinent because many substances have the capacity of splitting hydrogen peroxid into molecular oxygen and water. Furthermore, catalytic effect of catalase is apparently destroyed by the decomposition of hydrogen peroxid (Morgulis, 1921; Heinicke, 1924), but the products, water and molecular oxygen, are not considered as being toxic. It is possible, however, that the accumulation of certain substances used by the manufacturers in the preservation of the hydrogen peroxid might become toxic to the catalase. In the case of the fruits of the pear, however, it does not appear that the decomposition of hydrogen peroxid results from the action of a thermo-stable inorganic catalyst, since boiling of the pulp destroys its power to act upon hydrogen peroxid. It therefore would seem that the catalase was colloidal in nature, either as an organic substance or as a colloidal form of some metal. In either case it may be permissible to consider catalase as a product or by-product of protoplasmic activities, and as enzyme-like in nature.

The boiling of the preparation drove out some of the dissolved gases which were reabsorbed during the agitation, resulting in a slight negative pressure. The preparations boiled for 30 minutes absorbed about twice as much gas as those boiled 15 minutes. The addition of excess CaCO_3 indicated very slight evolution of a gas, in all probability carbon dioxide, from the effect of the fruit acids upon the CaCO_3 . With the preparations that were permitted to stand 24 hours before the determinations were made, the absence of CaCO_3 resulted in a greater negative pressure and the addition of excess CaCO_3 in a greater positive pressure than when there was no delay in making the tests.

The absorption as measured might also have been due to the oxygen taken up by the catechol-like compound in the fruit pulp and juice which behaved as pyrogallol (Overholser and Cruess, 1923).

Determination of H-ion Concentration

The hydrogen-ion determinations were made electrometrically. The hydrogen was furnished by a cylinder of the compressed gas which bubbled through an alkaline solution of pyrogallol, soda-lime tower, Orsat KOH solution, and water, respectively, before passing into the hydrogen electrode. The electrodes were standardized from time to time by using $M/20$ KH phthalate (potassium acid phthalate) solution at 20°C ., and thus obtaining an E. M. F. reading of 0.480 or a pH of 3.97.

EXPERIMENTAL DATA

The Correlation Between Catalase Activity and Hydrogen-ion Concentration in Certain Varieties of Pears

It was noted in preliminary tests that the catalase activities as well as the pH values of different varieties of pears varied. An attempt, therefore, was made to determine for certain pears the varietal characteristic in this respect, and to see if any correlation existed between catalase and the pH value of the varieties.

The fruits used had been harvested in the fall and retained in storage at 0° C. until the determinations were made the following spring. The data presented in table 1 indicate that the catalase activity of pears differed widely depending upon the variety. Of the European pears studied, the Wilder appeared to have the most active catalase and the Pound variety the least active.

The addition of excess calcium carbonate increased the catalase activity of all the varieties. The same relative order of catalase activity, however, was shown with and without calcium carbonate. Hence only the data without calcium carbonate are presented.

There appeared to be some correlation of catalase activity with the pH value of the tissue since those varieties highest in pH value were, on the average, highest in catalase activity, and *vice versa*. The data thus agree with Harvey (1920), Weiss and Harvey (1921), and others.

TABLE 1. *The Average pH Value and Catalase Activity of Pear Varieties (Without Addition of CaCO₃)*

Variety	No. of Tests Averaged	Average pH Values	Average cc. of Oxygen Evolved per Minute
Wilder.....	6	5.36	50.6
Winter Nelis.....	30	4.44	50.2
Easter.....	16	4.50	49.0
Bartlett.....	26	4.46	44.8
Seckel.....	28	4.43	42.4
Forelle.....	32	4.21	18.8
Hardy.....	28	4.15	15.6
Vicar.....	4	4.11	10.6
Barry.....	24	4.08	8.4
Pound.....	26	3.79	1.0

The Effect upon the Catalase Activity of Modification of pH Value by Adding N/10 NaOH and HCl

Merl and Daimer (1921) stated that the optimum hydrogen-ion concentration for flour catalase extended from pH 6.2 to an alkaline reaction. Morgulis (1921) found that the optimum H-ion concentration for the catalase reaction was pH 7.0, but the reaction at pH 6.4 was 98 percent complete, so that experiments should be conducted in a medium slightly

on the acid side (pH 6.7 to 6.9), thus preventing spontaneous decomposition of the hydrogen peroxid which occurred most rapidly at pH 7.0 and above. Rona and Damboviceanu (1922) found the optimum activity of liver catalase ranged rather widely around pH 7.0; fair activity was observed nearly up to pH 11.0, at which concentration it was markedly retarded.

First Experiment. Louise pears harvested August 25, and stored at 0° C., were utilized April 21. The standard method required the addition of 100 cc. of distilled water, and as the tenth-normal NaOH or HCl was added the water was correspondingly reduced so as to keep the volume the same and prevent dilution.

The data shown in table 2 indicate that with the mature Louise pears the lowering of the pH value of the medium from 4.62 to 3.95 reduced the catalase activity nearly three-fourths. When the pH was lowered to 3.37, the catalase was less than one-eleventh as active; and with pH values of 3.25 and 3.12 the catalase activity was inhibited.

The raising of the pH to from 4.77 to 5.51 approximately doubled the catalase activity. The further raising of the pH value by the addition of tenth-normal NaOH apparently tended again to decrease the catalase activity until with a pH value of 8.41 the activity was reduced to about the same as the check.

TABLE 2. *The Effect of Modification of the pH Value upon the Catalase Activity of Louise Pears (Average of Three Determinations)*

Treatment	pH Value	Average cc. of Oxygen Evolved per Minute
None (check).....	4.62	15.0
10 cc. N/10 HCl added.....	3.95	4.2
20 cc. N/10 HCl added.....	3.37	1.3
30 cc. N/10 HCl added.....	3.25	0.03
40 cc. N/10 HCl added.....	3.12	0.11
None (check).....	4.57	14.3
10 cc. N/10 NaOH added.....	4.77	28.5
20 cc. N/10 NaOH added.....	5.51	27.0
30 cc. N/10 NaOH added.....	6.42	23.5
40 cc. N/10 NaOH added.....	8.41	15.1

Second Experiment. Clairgeau pears, harvested in September 1924, were utilized from 0° C. storage in May 1925. The data are averaged in table 3.

The raising of the pH value by the addition of tenth-normal NaOH from 5.13, the average of the check, to from 6.4 to 6.93 gave the maximum catalase activity. Further increase in the pH value to 8.73 reduced the activity below the maximum but still favored greater activity than the check. Increasing the pH value to 9.41, however, reduced the catalase below the check.

TABLE 3. *The Effect of Modification of pH Value upon the Catalase Activity of Clairgeau Pears (Average of Four Experiments)*

Treatment	pH Value	Average cc. of Oxygen Evolved per Minute
None (check).....	5.13	14.0
10 cc. <i>N</i> /10 HCl.....	4.16	3.0
20 cc. <i>N</i> /10 HCl.....	3.31	0.13
30 cc. <i>N</i> /10 HCl.....	2.77	0.08
40 cc. <i>N</i> /10 HCl.....	2.36	0.3
10 cc. <i>N</i> /10 NaOH.....	6.40	20.0
20 cc. <i>N</i> /10 NaOH.....	6.93	19.3
30 cc. <i>N</i> /10 NaOH.....	8.73	16.3
40 cc. <i>N</i> /10 NaOH.....	9.41	11.5
50 cc. <i>N</i> /10 NaOH.....	10.60	9.4

On the other hand, the lowering of the pH value from 5.13 of the check to 4.16 reduced the catalase activity to less than one-fourth that of the check, and pH values of 3.31 and lower permitted no measurable catalase activity. The results in general agree with those obtained with the Louise variety.

Third Experiment. Since the foregoing experiments indicated that an increase of the pH value by the addition of as much as 40 cc. of tenth-normal NaOH reduced the catalase activity, an experiment was conducted with Fox pears to determine the effects of the addition of larger amounts of NaOH. The data are shown in table 4.

While 60 and 80 cc. of tenth-normal NaOH were added, because of the lower original pH value of the Fox, the pH values were not greatly increased over those previously obtained. With the highest pH value, however, of 9.72, the catalase was greatly reduced but was still active. The Fox responded to the lowering of the pH value about as would be expected from data obtained with other varieties. With pH values of 3.93 and lower there was no catalase activity. In fact, with the lower pH values there developed a measurable negative pressure.

TABLE 4. *The Effect of Modification of pH Value upon Catalase Activity of Fox Pears (Average of Four Determinations)*

Treatment	pH Value	Average cc. of Oxygen Evolved per Minute
None (check).....	4.41	2.9
10 cc. <i>N</i> /10 HCl.....	3.93	-0.16
20 cc. <i>N</i> /10 HCl.....	3.59	-0.20
40 cc. <i>N</i> /10 HCl.....	2.83	-0.55
20 cc. <i>N</i> /10 NaOH.....	5.48	4.3
40 cc. <i>N</i> /10 NaOH.....	6.71	9.65
60 cc. <i>N</i> /10 NaOH.....	9.10	2.6
80 cc. <i>N</i> /10 NaOH.....	9.72	0.9

The Relation of Maturity When Harvested to Catalase Activity of the Fruits of the Pear

Reed (1916) found that in the juice of the pineapple the catalase increased during the ripening of the fruit.

To determine the effect of degree of maturity upon the catalase activity, pickings were made at intervals from May 28 until August 24. The varieties employed for the purpose were the Easter Beurre, Forelle, Winter Nelis, Seckel, Bartlett, Pound, Barry, and Hardy. The average weight in grams, the pH value, and the catalase activity were obtained. The data summarized in table 5 indicate that in each variety the catalase decreased markedly as maturity advanced.

TABLE 5. *The Effect of Maturity when Harvested upon the Catalase Activity and the pH Values of Pear Varieties (Without CaCO₂)*

Variety	Date Harvested 1925	Average Wt. per Specimen (grams)	pH Value	Average cc. of Oxygen Evolved per Minute
Winter Nelis...	May 28	7.9	4.67	94.6
	June 6	10.3	4.59	90.0
	June 12	14.5	4.67	82.6
	June 25	30.0	4.47	68.4
	July 9	40.0	4.38	41.4
	July 18	44.1	4.41	30.0
	July 27	47.1	4.40	31.4
	August 4	76.2	4.41	31.8
	August 15	77.0	4.37	26.4
	August 24	76.5	4.39	17.4
Seckel.....	May 28	5.6	4.89	100.0
	June 12	11.9	4.50	64.4
	June 25	19.3	4.53	50.2
	July 9	27.7	4.45	37.2
	July 18	33.0	4.53	36.0
	July 27	33.7	4.50	33.2
	August 4	49.0	4.25	19.8
	August 15	60.0	4.26	16.2
	August 24	64.0	4.26	15.2
Barry.....	June 6	13.3	4.49	106.0
	June 12	23.2	4.42	86.0
	June 25	32.1	4.30	84.4
	July 9	32.1	4.12	19.8
	July 18	41.4	4.09	15.4
	July 27	63.3	4.17	4.6
	August 4	98.2	4.04	4.0
	August 15	120.2	4.10	4.0
	August 24	145.1	3.93	0.1
Pound.....	June 6	56.2	3.94	6.40
	June 12	62.7	3.93	0.70
	June 25	80.0	3.85	0.34
	July 9	109.4	3.81	0.16
	July 18	128.6	3.75	0.20
	July 27	214.0	3.80	0.20
	August 4	231.2	3.73	0.08
	August 15	252.1	3.78	0.40
	August 24	260.0	3.70	0.20

TABLE 5.—*Continued*

Variety	Date Harvested 1925	Average Wt. per Specimen (grams)	pH Value	Average cc. of Oxygen Evolved per Minute
Easter.....	June 12	30.8	4.83	125.0
	June 25	51.9	4.50	104.0
	July 9	74.6	4.49	80.6
	July 18	89.0	4.52	42.4
	July 27	119.6	4.43	14.2
	August 4	176.6	4.41	13.0
	August 15	180.0	4.37	6.2
	August 24	182.0	4.31	6.2
Hardy.....	May 28	14.5	4.64	80.0
	June 12	39.2	4.31	35.6
	June 25	45.6	4.15	18.8
	July 9	76.6	4.04	4.8
	July 18	96.6	3.93	1.0
	July 27	115.0	3.98	1.8
	August 4	114.5	3.98	1.0
	August 15	116.0	4.04	0.6
	August 24	116.5	4.09	0.1
Bartlett.....	May 28	15.2	4.76	104.0
	June 6	22.2	4.60	76.6
	June 12	30.3	4.46	74.0
	June 25	55.4	4.40	32.0
	July 9	85.0	4.35	32.0
	July 18	91.0	4.40	24.4
	July 27	113.0	4.37	8.6
	August 4	103.4	4.37	8.4
Forelle.....	May 28	7.0	4.55	56.0
	June 6	14.4	4.40	48.0
	June 12	18.5	4.34	50.6
	June 25	29.3	4.21	31.6
	July 9	57.7	4.24	9.4
	July 18	51.3	4.25	4.6
	July 27	93.0	4.01	1.6
	August 4	70.6	4.09	0.68
	August 15	80.1	4.09	0.46
	August 24	85.8	3.90	0.20

The pH value also became somewhat lower as the maturity of the fruit advanced and this indicated a correlation between the decline in catalase activity and the lowering of the pH value of the fruit. This lowering seemed comparatively slight but it may have been at the critical point, since the lessening of the catalase activity was marked.

The gradual increase in hydrogen-ion concentration as the pears approached maturity appeared to agree with the studies of Bigelow and Gore (1905), who found that the percentage of total acids in peaches increased as the fruit matured on the tree from the time between the June drop and the time of market ripeness.

These changes in the catalase activity of the pear with advance in maturity did not agree with the findings of Reed on the juice of the pineapple.

The greater catalase activity may be correlated with greater vegetative activity of the young fruits, and thus agree with Heinicke's finding (1924) that catalase activity was generally greater under conditions favoring vegetative growth. It is probable also that respiratory intensity was greater with the younger fruits and that this might have coincided with the greater catalase activity. Overholser (in unpublished data) found that the respiration intensity of immature pears was much higher than that of more mature fruit.

There may, however, have been some relationship between catalase decline and increase in size of the fruit. The initial average weight per specimen of the varieties was about eight percent of the final average weight. The catalase activity at maturity was about six percent of that when the first pickings were made.

The Effect of Previous Storage Temperature Upon Catalase Activity

Magness and Burroughs (1921-22) found that Baldwin and Winesap apples previously stored at 0° C. were distinctly lower in catalase activity than fruit stored at 1.7° C., and that this, in turn, was lower than that from the higher temperature of the cellar storage. There was an increase in catalase in the fruit following its removal from cold storage to 18.3° C., which was relatively greater in fruit held at 0° C. than in fruit held at a slightly higher temperature throughout the season, and was such that the final catalytic activity after 16 to 18 days at 18.8° C. was about the same regardless of previous storage condition. That the catalase apparently was not directly related to temperature, as such, was shown by removing Baldwins from 18.3° C. storage, where high catalase activity had developed, to 0° C. storage. After periods up to two months in the 0° C. storage there was no apparent decrease in catalase activity.

The effect of the storage temperature upon the catalase activity of pears was determined in the first four experiments by using fruit previously stored at 0° C. for some time. In the last five experiments fruit was used immediately after harvest.

First Experiment. Louise pears, harvested August 26, and stored at 0° C., were removed February 1 and portions placed in temperatures of 21° C. and 28° C. for a period of 14 days. As a check a sample was also kept at 0° C.

The catalase determinations were made with an excess of CaCO_3 added. At the time the determinations were made the fruit from 0° C. was ripe, that from 21° C. soft ripe, and that from 28° C. soft and wilted but apparently not as ripe as the fruit kept at 21° C. (Overholser and Taylor, 1920). The data are shown in table 6.

TABLE 6. *The Effect of Storage Temperature upon Catalase Activity of Louise Pears (Average of Three Determinations)*

Storage Temperature	Total cc. of Oxygen Evolved per Minute
0° C. continuously.....	27.2
0° C. 5 months; 21° C. 14 days.....	35.4
0° C. 5 months; 28° C. 14 days.....	48.0

Fruit removed from 0° C. and kept at the higher temperatures of 21° C. and 28° C. for a period of two weeks preceding the test exhibited greater catalase activity than fruit kept continuously at 0° C.

Second Experiment. The effect of storage temperature was further studied, using Forelle pears harvested in the fall and kept at 0° C. On February 16, lots were removed to each of the following temperatures: (a) 18°–21° C.; (b) 27° C.; (c) 73° C. One lot was also retained at 0° C. as a check. Catalase determinations with the addition of CaCO₃ were made after 72 and 192 hours storage. The data are given in table 7.

TABLE 7. *The Catalase Activity of Forelle Pears Stored at 0° C. for Five Months as Influenced by Short Exposures to Higher Temperatures (Average of Six Determinations)*

Temperature of Storage Preceding Test	Average cc. of Oxygen Evolved per Minute
Stored at 0° C. continuously.....	26.6
Removed to 18°–22° C. for 72 hours.....	25.8
Removed to 18°–22° C. for 192 hours.....	48.0
Removed to 27° C. for 72 hours.....	44.0
Removed to 27° C. for 192 hours.....	54.2
Removed to 73° C. for 72 hours.....	1.0
Removed to 73° C. for 192 hours.....	0.0

It appeared that Forelle pears at the intermediate temperatures of 21° C. and 28° C. exhibited the greatest catalase activity. Furthermore, within the limits of the test, the longer the period of time the fruit was kept at these temperatures the greater the increase in catalase activity. At the highest temperature (73° C.), however, after an interval of 72 hours, the catalase was nearly destroyed and after 192 hours was quite destroyed.

Third Experiment. An experiment was conducted to determine the effect of relatively long storage at 20° C. upon the catalase activity of fruit previously stored at 0° C. On March 15, Forelle pears were removed from storage at 0° C. and placed at 20° C. for three weeks, or until the fruit was well softened and much over-ripe. As checks, determinations were made of the catalase activity of fruits at 0° C. both at the beginning and close of the experiment. The data are shown in table 8.

TABLE 8. *The Effect of Prolonged Storage at 20° C. as Compared With Continuous Storage at 0° C. upon Catalase Activity (Average of Six Determinations)*

Temperature of Storage	Average cc. of Oxygen Evolved per Minute
Check: 0° C. (beginning of experiment).....	25.0
Check: 0° C. (close of experiment).....	26.0
20° C. (after 21 days).....	4.3

Storage of Forelle pears at a temperature of 20° C. for three weeks or until the fruits were over-ripe resulted in a decrease in catalase activity as compared with fruit kept continuously at 0° C.

Fourth Experiment. The effect of storage at a temperature below 0° C. upon the catalase activity of the Forelle were determined. Fruit removed from 0° C. storage and placed in -10° C. storage was kept rigidly frozen for two months. The data are summarized in table 9.

TABLE 9. *The Effect of Prolonged Freezing upon the Activity of Forelle Pears (Average of Eight Determinations, Two Separate Lots)*

Storage Temperature	Treatment	pH Value	Average cc. of Oxygen Evolved per Minute
0° C.....	No CaCO ₃	4.74	11.7
0° C.....	CaCO ₃ added	6.95	30.8
-10° C.....	No CaCO ₃	4.67	1.54
-10° C.....	CaCO ₃ added	6.95	11.8

The data show that storage for a period of two months at the temperature of -10° C. reduced the catalase activity in the Forelle pear, as contrasted with the fruit stored at 0° C. The pH value of the fruit was also somewhat lowered by the freezing. Stoland and Walling (1921) found that blood catalase was partly destroyed at -14° C.

Fifth Experiment. The preceding experiments have shown the responses of the catalase to a change of storage temperature upon pears previously stored at 0° C. for some four or five months. The effect of storage temperature upon catalase activity of pears just harvested and not previously subjected to storage temperatures was studied. It is of interest to point out that Burroughs (1923) found that Wagener and Baldwin apples picked while immature and stored for a period of 4 to 57 days at 0° C. respired more rapidly when placed at 20° C. than did apples placed at 20° C. immediately after harvest.

Young fruits of the Chadborne variety were harvested June 26 in a hard, green, and unripe condition with abundant starch distributed throughout the tissues. The catalase activity was determined at harvest time. The fruit was then placed at 0° C. and samples withdrawn for further catalase determinations after 11 and 17 days storage. The data are given in table 10.

There was no appreciable increase in the catalase activity of the young fruits of the Chadborne pear after 11 days storage at 0° C. After 17 days storage, however, the catalase activity had greatly increased. There was a gradual increase in the pH value from 4.15 when harvested to 4.25 after eleven days storage, and to 4.35 after seventeen days storage at 0° C.

TABLE 10. *The Effect of Storage at 0° C. after Harvest upon Catalase Activity (Average of Six Determinations)*

No. of Days Stored at 0° C.	Treatment	pH Value	Average cc. of Oxygen Evolved per Minute
None.....	No CaCO ₃	4.15	14.2
None.....	Excess CaCO ₃	7.00	29.5
11.....	No CaCO ₃	4.25	14.3
11.....	Excess CaCO ₃	7.00	30.1
17.....	No CaCO ₃	4.35	20.2
17.....	Excess CaCO ₃	6.40	48.3

Sixth Experiment. The increase in catalase activity with storage at 0° C. was of interest, and hence another experiment was conducted to demonstrate further this change, as well as those resulting from storage at other temperatures.

Easter Beurre pears were harvested on August 28 and approximately 1,000-gram samples were placed at each of the following temperatures: 40° C.; 30° C.; 20° C.; 7.5° C.; 0° C.; and - 12° C. The fruit remained at these temperatures for 7 days. Catalase determinations were made immediately after harvest and at the end of the storage period.

After one week's storage at 40° C. the epidermis of the fruits had browned considerably, and the flesh likewise had become brownish in color, especially about the core. The tissue immediately below the epidermis, however, possessed a greenish color. The texture had become slightly softened, possibly due to wilting. The fruits at 30° C. were firm in texture, the epidermis was green, and the flesh was white with a greenish layer just beneath the epidermis. The fruits at 20° C., 7.5° C., and 0° C., were very firm; the epidermis was green, and the flesh a clear white color throughout. The fruit at - 12° C. were frozen rigidly. The epidermis was green and the flesh a clear white color throughout.

The data in table 11 indicate the changes during the one week's storage as contrasted with the catalase when harvested. There was an increase in catalase activity as a result of storage at 0.0° C. and at - 12° C. The change in catalase activity at 20° and 7.5° C. was slight. The increase in activity at 30° C. was appreciable and at 40° C. was quite marked. The addition of CaCO₃ did not greatly affect the relative responses except that all of the determinations had higher values.

In this connection it is of interest to note that Magness and Diehl (1924) suggest that at 0° C. the respiration of apples involves approximately equal oxidation of acids and sugars; at 4.5° and 16° C. sugars are mainly oxidized; and at 30° C. there is a somewhat greater oxidation of acids.

TABLE 11. *The Catalase Activity of Easter Beurre Pears as Influenced by One Week's Storage at Different Temperatures (Average of Four Determinations)*

Storage Temperature	Treatment of Preparation	pH Value	Average cc. of Oxygen Evolved per Minute
Before storage.....	No CaCO ₃	4.39	3.10
Before storage.....	CaCO ₃ added	6.66	22.2
40° C.....	No CaCO ₃	5.97	34.1
40° C.....	CaCO ₃ added	6.98	50.0
30° C.....	No CaCO ₃	4.82	16.6
30° C.....	CaCO ₃ added	6.82	33.3
20° C.....	No CaCO ₃	4.52	6.0
20° C.....	CaCO ₃ added	6.85	17.4
7.5° C.....	No CaCO ₃	4.33	3.25
7.5° C.....	CaCO ₃ added	6.73	25
0° C.....	No CaCO ₃	...	11.87
0° C.....	CaCO ₃ added	...	36.6
-12° C.....	No CaCO ₃	...	11.5
-12° C.....	CaCO ₃ added	...	33.3

Seventh Experiment. Of the temperatures employed, fruit could be kept in fresh edible condition for a long period of time only at 0° C. At room temperature and above the fruit became over-ripe, rotted, or shriveled within approximately three weeks. At 7.5° C. the storage period was from four to six weeks, depending upon the variety and degree of maturity when harvested. At -12° C., the fruit was quickly frozen rigidly and upon thawing became rapidly soft, mushy, watery, and darkened in color.

Four varieties—the Hardy, Forelle, Seckel, and Pound—were stored. Under favorable conditions, these could be kept for six months at 0° C. and, when harvested, showed a rather wide difference in catalase activity and pH value.

The data in table 12 give the pH and catalase activity when harvested and after six months storage at 0° C., and indicate that with prolonged storage at 0° C. the catalase activity may greatly increase over the initial activity when harvested. The lower the catalase activity at the beginning apparently the greater is the increase with storage.

TABLE 12. *A Comparison of the Catalase Activity of Pears at Harvest and after Six Months Storage at 0° C.*

Variety	pH Value:		Av. cc. of Oxygen Produced per Minute:	
	When Harvested	After 6 Months Storage	When Harvested	After 6 Months Storage
Hardy....	4.09	4.67	0.24	10.0
Forelle...	3.50	4.74	0.20	11.6
Seckel....	4.26	4.67	15.20	23.6
Pound....	3.70	4.41	0.16	5.4

With the four varieties studied there appeared to be some correlation between catalase activity when harvested and the pH value. This correlation was not so consistent after six months storage at 0° C. although the pH value had increased in every case with storage. The average pH value of the four varieties when harvested was 3.99, and after storage 4.62.

Eighth Experiment. Vicar pears were harvested July 20, and stored at 40°, 30°, 20°, 7.5°, 0°, and - 12° C. Catalase determinations were made after 7, 14, and 22 days storage, respectively.

The fruits were not fully mature when harvested, as the iodine test showed the presence of a large amount of starch distributed throughout the flesh, with the exception of only small amounts in the core area or in the stone cells. The ferric chlorid test also indicated the presence of a relatively large amount of tannin in the flesh, with the exception of the stone cells, which appeared to have no tannin. The vascular bundles likewise had small amounts of tannin. The greatest amount, as indicated by the intense dark brown color, appeared to be in the core.

After 14 days storage at 40° C. the fruits had wilted to such a degree that the tissue was pliable in texture. The epidermis had greatly shrivelled but was green in color. The flesh lacked characteristic flavor, and the starch had completely disappeared. The presence of tannin throughout the flesh, however, was demonstrated distinctly. After 22 days storage at 40° C. the fruit had wilted to such an extent that the tissue appeared to be almost desiccated. The epidermis and pulp had become brown to black throughout.

After 14 days the epidermis of the fruit stored at 30° C. had acquired a slight yellowish tinge. The tissue was white and somewhat juicy, although appreciable water had been lost, as was evidenced by the leathery texture. The starch had disappeared and there appeared to be slightly less tannin present than in the fruit kept at 40° C. After 22 days storage the epidermis had lost its greenish color and acquired a yellowish color. The tissue had lost additional moisture, and the texture was tough and the flavor insipid.

The fruits kept at 20° C. were fairly firm in texture after 14 days storage. The epidermis had become somewhat lighter green in color than when harvested. The starch had disappeared, and the tannin was likewise present in less amount than in the fruit stored at 30° C. The color of the epidermis was still green after 22 days at 20° C. but the fruits had wilted considerably more.

The fruit stored at 7.5° C. for 14 days remained firm and green. The flesh was white and rather juicy with a sweetish flavor. There was still some starch present in the periphery just beneath the epidermis. Tannin was present in abundance throughout. The fruit remained firm and green after 22 days at 7.5° C. The starch had disappeared but the tannin remained.

Of the fruit stored at 0° C. for 14 days the texture was very firm, the

epidermis green in color, the flesh white, and the flavor insipid. The maturity was less advanced than at 7.5° C., the starch being present except in the core region. After 22 days storage the starch was present in the peripheral portion for a depth of approximately one-fourth of an inch. Tannin was present, about as initially found, throughout the storage period.

The fruit at -12° C. remained frozen rigidly throughout during the entire storage period with no visible changes in maturity.

Ninth Experiment. A similar experiment was conducted simultaneously with the Chadborne variety of pears. The ripening changes in the Chadborne at the different temperatures were about as described for the Vicar. The Chadborne, however, was further advanced in maturity when harvested than was the Vicar and was sufficiently ripe for commercial harvest. The method of procedure was identical and the response to the storage at different temperatures closely followed that of the Vicar.

The data in table 13 indicate that the duration of the storage period had a greater or less effect upon the catalase activity. It appeared that Blackman's (1905) time factor became operative.

TABLE 13. *The Effect of Duration of Storage Period at Different Temperatures upon Catalase Activity (Average of Vicar and Chadborne Pears, Eight Determinations)*

Storage Temperature	No. Days Stored	Average pH Value	Average cc. of Oxygen Evolved per Minute
Not stored.....	0	4.06	4.4
40° C.....	8	4.56	23.4
40° C.....	14	4.91	15.7
40° C.....	22	...	12.5
30° C.....	8	4.22	9.9
30° C.....	14	4.29	6.5
30° C.....	22	4.45	11.1
20° C.....	8	4.05	5.5
20° C.....	14	4.15	3.6
20° C.....	22	4.14	2.1
7.5° C.....	8	4.05	4.0
7.5° C.....	14	4.11	3.0
7.5° C.....	22	4.00	0.9
0° C.....	8	...	10.6
0° C.....	14	4.16	11.0
0° C.....	22	4.19	17.7
-12° C.....	8	...	18.4
-12° C.....	14	4.25	10.7
-12° C.....	22	4.06	3.1

Storage at -12° C. and at 40° C. for 8 days resulted in an increase in catalase activity, but storage for 14 days gave a reduced catalase activity,

and storage for 22 days gave such a further reduction that at -12°C . the activity was less than the initial amount when harvested. The reduction was not so marked at 40°C .

The data indicated that with relatively short periods of storage at -12°C . there was an accelerating effect; but that with further storage an injury resulted which was marked. Since the fruit used was relatively immature, in all probability the degree and rapidity of injury was not as great as would have resulted if the pears had been well matured (de Villiers, unpublished data). Furthermore, Carrick (unpublished data) found that as a result of freezing there was an initial increase in the rate of respiration which, with continued freezing as further injury resulted and a greater proportion of the cells were killed, fell off. It seems possible, therefore, that the first marked increase in catalase activity of fruit stored at -12°C . for 8 days may have coincided with increased respiration, and the reduction in catalase activity after 14 days with a gradually decreasing respiratory intensity; and that the marked drop in catalase activity after 22 days at -12°C . was related to the death of a large proportion of the cells.

Carrick (1924), working with apples, stated that it was probable that an interaction of the solute ions and ionogenic colloids of the protoplasm occurred during or immediately after severe freezing. The ionizable colloids of the cell, such as proteins, were flocculated. This, and the adsorption of solute ions by the precipitated colloids of the cytoplasm, might be expected to exert a profound effect on the catalase activity of frozen pear tissue.

Storage at 0°C . and at 30°C . tended to result in a gradual increase during the total storage period of 22 days. After six months storage at 0°C . the catalase was appreciably greater than when harvested. The increase at both temperatures was apparently correlated with changes which resulted in higher pH values. This increase in pH value agreed with the work of Bigelow and Howard (1905) who found with five varieties of peaches stored at 0°C . for from 28 to 63 days that the acids decreased. It was possible that the increase at 30°C . might be correlated with greater respiratory intensity, but this did not seem a probable cause for the greater increase in catalase activity with 22 days storage at 0°C ., although the findings of Hopkins (1924) were significant in this connection.

As contrasted with the fruit removed from 0°C . to 20°C ., the pears stored at 20°C . after harvest gave only a slight increase after 8 days and a gradual decrease with 14 and 22 days storage.

The striking effect of storage temperature upon catalase activity was that obtained at 7.5°C ., where there was a continuous decrease in the activity during the storage period of 22 days. In connection with the low catalase activity as a result of storage at 7.5°C ., as contrasted with storage at 0°C . and the other temperatures, it is of interest again to refer to the work of Hopkins (1924) who found with potatoes that respiration was

accelerated at 0°C ., and that the respiratory intensity for some time was greater than at 4.5°C .

The respiratory intensity reached a minimum at 4.5°C . Hopkins found also that sugar accumulated in potatoes at 0°C . and began to disappear rapidly when stored at 4.5°C ., and he suggested that the acceleration of respiration at 0°C . was dependent on the changing concentration of sugar. It is of interest to point out that with the pears employed the fruit was relatively immature and hence starch was abundantly present to begin with. There may have been a sugar relationship with the temperatures 7.5°C . and 0°C . similar to that which Hopkins found with the potato.

There also appeared to be a possibly significant correlation between the effect of the various temperatures upon catalase activity and the changes in pH value, in that the pH value tended to be higher at the storage temperatures which resulted in increase of catalase activity and lower at the temperatures which resulted in decrease of catalase activity. The pH values, however, were somewhat incomplete.

SUMMARY AND CONCLUSIONS

1. The evidence indicated that the catalase activity of the pear tissue could be measured most accurately at a temperature of 15°C . When temperatures above 20°C . were employed there was a measurable spontaneous decomposition of the hydrogen peroxid.

2. The oxygen evolved by the catalase of pear pulp with the amounts of material employed was almost directly proportional to the quantity of hydrogen peroxid used.

3. Increasing the amount of pear pulp increased the oxygen evolved from a given quantity of hydrogen peroxid, but the relative increase was not as great as when the amount of hydrogen peroxid was increased.

4. The addition of increasing amounts of water reduced the oxygen evolved within a given period of time.

5. Boiling the pear pulp for fifteen minutes destroyed the catalase activity.

6. The varietal catalase activities and pH values of the pulp of ten varieties of pears were studied and high pH values appeared to coincide with high catalase activities.

7. With the varieties Louise, Clairgeau, and Fox, when the pH of the reacting pulp was reduced to approximately 3.55 by the addition of tenth-normal HCl, the catalase was almost completely checked.

8. As the pH value was raised by the addition of tenth-normal sodium hydroxid the catalase activity increased to a maximum with a pH of from 6.00 to 6.50. This indicated that with pear pulp the maximum activity was slightly on the acid side rather than at neutrality or under slight alkaline conditions such as would be effected by the addition of excess calcium carbonate.

9. The catalase activity remained greater than the check until the pH was higher than 8.50.

10. As the pH of the reacting pulp of the Fox variety was raised much above 9.00 the catalase activity was again lowered, and at a pH close to 10.00 the activity was almost inhibited.

11. Freezing at -10° C. for two months greatly decreased the catalase activities as compared with storage at 0° C.

12. The effect of the storage temperature upon catalase activity depended upon the duration of the storage period, which varied with the temperature.

13. Storage at -12° C. and 40° C. for eight days increased the catalase activity, but the activity decreased with 14 and 22 days storage.

14. Storage at 0° and 30° C. resulted in a gradual increase in catalase activity throughout the storage period of 22 days.

15. Storage at 20° C. gave a slight increase in catalase activity after 8 days storage, and then a gradual falling off after 14 and 22 days.

16. Storage at 7.5° C. resulted in a continuous decrease throughout the storage period of 22 days.

17. The average pH value tended to be higher at the storage temperatures resulting in catalase increase and lower where the catalase decreased.

18. When pears that had been stored previously at 0° C. for some time were brought to higher temperatures of 20° to 28° C. for periods of 3 to 14 days the catalase activity seemed to be greater. Storage for three weeks, however, at 20° C. resulted in a decrease in catalase activity.

19. Storage at 28° C. for from 3 to 14 days increased the catalase to a greater degree than did storage at 20° C.

20. Storage at 72° C., however, for 3 days resulted in a marked reduction in catalase activity, and after 8 days the catalase activity was destroyed.

21. Storage at 0° C. for six months with four varieties resulted in an increase in catalase activity and in pH value over that present when harvested.

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THE MORPHOLOGY AND ANATOMY OF THE FLOWERS OF THE SALICACEAE I

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The family Salicaceae, comprising the two genera *Salix* and *Populus*, is, with some few exceptions, placed by taxonomists at or near the base of the Amentiferae, that anomalous, heterogeneous assemblage of so-called primitive dicotyledonous angiosperms. The uncertainty as to the status of the Amentiferae as a whole is reflected also in the shifting position of the Salicaceae, which within the past century and a half have run the entire amentiferous gamut from the lowest position in the classification of de Jussieu the elder (25),¹ in 1759, to the highest in that of Eichler (16), in 1878. In addition to this nomadic life within the confines of the Amentiferae itself, the Salicaceae have in a few instances even wandered without the fold. In 1867, Parlatore (27), on structural grounds, divorced them completely from the amentiferous alliance and placed them as a separate and distinct family between the Amentaceae on the one hand and the Urticaceae on the other. More recently also there has been a growing tendency on the part of a few students to accord a relatively high rank to the family. Among those who have so placed the Salicaceae are Hallier (19) who, in 1912, placed the group in the order Passionales between the Euphorbiaceae and the Passifloraceae; and Bessey (11) who assigned the Salicaceae to the order Caryophyllales between the families Tamaricaceae and Podostemonaceae.

Differences of opinion in regard to the nature of the inflorescence and flower of the Amentiferae are responsible, in a measure, for the varying positions assigned the group. The flower has been interpreted as primitively unisexual, dioecious, and naked, in process of evolution toward a type with differentiated calyx and conspicuous corolla. On the other hand, the opposite view has also been maintained—that bisexual, dioecious flowers with conspicuous floral envelopes, which have been reduced, was the primitive condition. Each view has been supported by distinguished botanists.

These two conflicting theories in regard to the flower of the Amentiferae in general apply in particular to the floral structure of the Salicaceae. Adherents of the one, with morphological, paleontological, and, more recently, histological evidence in hand, maintain that the family, if not the most primitive, is at least correctly placed at or near the beginning of the

¹ References are to literature cited in the bibliography appended to the second paper of this series, to appear in the following issue of the JOURNAL.

dicotyledonous line. Supporters of the second view, with evidence likewise derived from morphology, paleontology, and histology, assert with equal conviction that the family is highly evolved, its apparent primitive characters being due to simplification and reduction of parts.

OBJECT OF THE INVESTIGATION

In consideration of these opposed views, it was thought that an anatomical investigation of the salicacean flower might reveal additional evidence in support of one or the other theory. With this object as a goal, flowers of numerous species of *Salix*, representative of arctic, temperate, and tropical groups, and of several species of *Populus*, have been studied.

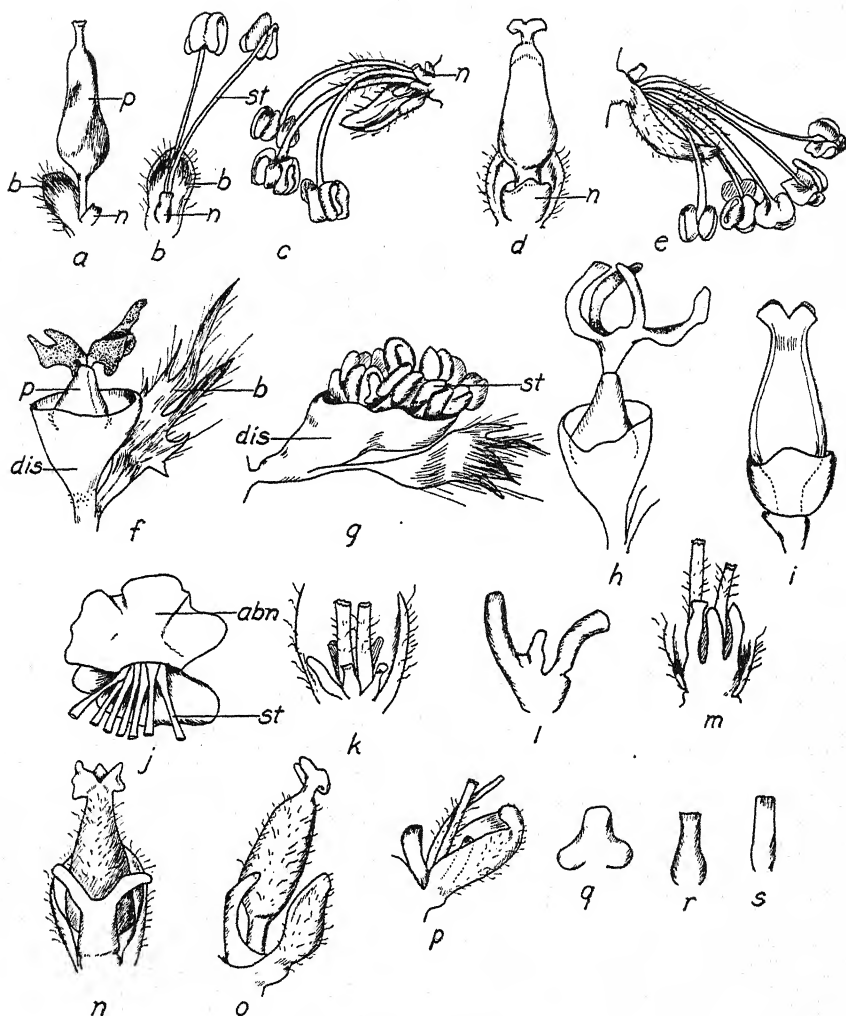
HISTORY OF THE FAMILY

Living Salicaceae are largely restricted to north temperate and arctic regions; the poplars are preëminently peculiar to the cooler part of the northern hemisphere (exception, subtropical *P. mexicana*); the willows are likewise an essentially arctic and north temperate group, though, due to greater adaptability to diversified climatic conditions, they have extended their range southward to, or else have survived in, subtropical and tropical regions, where, in South America, the group is represented by *S. Humboldtiana*, and in Africa by *S. safsaf*, *S. capensis*, etc.

Engler and Gilg give 18 as the number of existing species of poplars and 170 as that of existing species of willows. Berry (8) states that there are about 200 existing species of willows and about 25 poplars. The relative number of species of the two genera is strikingly reversed in their geologic history. There are about 125 fossil poplars, in addition to fossil forms identical with existing species. On the other hand only about half that number of fossil willows have been positively identified. 70 or 80 species of poplars are recorded from the upper Cretaceous and Eocene periods, after which there is apparently a reduction in number. The willows, in contrast, were represented by relatively few species in these earlier periods, but greatly increased in number in the Miocene, since which time they have persisted as a flourishing, plastic, up-grade group. *Populus primaeva*, discovered in the Cretaceous formation of Greenland, named and described by Heer, was for a long time the oldest known dicotyledon, and thus "served to suggest the primitive character of the Salicaceae as a whole among the Dicotyledons."

FLORAL STRUCTURE

In both genera the normally dioecious, apparently naked flowers are borne in pendulous or erect catkins, which in many cases mature before the foliage develops. The fertile flowers of *Salix* are borne solitarily in the axil of a bract, and each consists merely of a flask-shaped, stalked pistil accompanied by one or two small glands or nectaries (text fig. 1, a, d).



TEXT FIG 1. Habit sketches of salicacean flowers and floral parts: *a*, pistillate, and *b*, staminate flowers of a typical diandrous willow (*S. cordata*); *n*, nectary; *b*, bract; *p*, pistil; *st*, stamens. *c-e*, flowers of typical pleiandrous willows: *c*, *d*, *S. Bonplandiana*, and *e*, *S. Humboldtiana*. *f*, *g*, flowers of a typical poplar (*P. tremuloides*); *dis*, disk. *h-s*, disk (*h*, *Populus grandidentata*) and nectaries of salicacean flowers: *i*, adaxial view of cup-like nectary of *S. safsaf* ♀; *j*, basal part of staminate flower of *S. safsaf* with stamens pressed down in order to expose the broad, petaloid abaxial (*abn*) nectary; *k*, basal part of staminate flower of *S. reticulata* with whorl of nectaries surrounding the microsporophylls; *l*, lobed adaxial nectary of *S. reticulata* ♀; *m*, three-cleft adaxial nectary of *S. reticulata* ♂; *n* and *o*, adaxial and lateral aspects of Y-shaped nectary of *S. vestita* ♀; *p*, lateral aspect of nectaries of *S. Daviesii* ♂; *q* and *r*, beaker-shaped adaxial nectaries of staminate flowers of *S. cordata* and *S. longifolia*, respectively; *s*, typical cylindrical nectary characteristic of the more highly specialized forms of *Salix*.

The sterile flowers are likewise borne in the axils of bracts and are accompanied by one, two, or more small nectaries (text fig. 1, *b, c, e*).

The nectaries are exceedingly variable in size, shape, color, and number. In some species these glands are relatively large and conspicuous; in others they are small and inconspicuous. They may be flattened and petaloid (text fig. 1, *j*); Y-shaped (text fig. 1, *n*); beaker-shaped (text fig. 1, *q, r*); more or less cylindrical or cup-shaped (text fig. 1, *d*), etc.; and they may be notched, deeply lobed, or entire. Their color ranges from yellowish green through yellow and orange to a rich red (*S. amygdaloides*, *S. candida*). The usual number is one or two, but a higher number is frequently found, especially in tropical and arctic forms. When only one nectary is present, it is placed adaxially between the base of the sporophylls and the axis of the catkin; when two glands are present, the second occupies an abaxial position between the base of the sporophylls and the bract; when more than two occur, the nectaries are cyclically arranged in one or two whorls about the base of the sporophylls.

The bract consists of a single, usually entire scale, the size, shape, color, and persistency of which varies somewhat with the species (text fig. 1, *a-e*).

The fertile flowers of *Populus* are also borne solitarily in the axil of a bract, but the more or less ellipsoidal, sessile pistil arises from a "cup-shaped disk which is obliquely lengthened in front" (text fig. 1, *f, h*). The sterile flowers of *Populus* are each similarly subtended by a bract and also arise from a cup-shaped disk (text fig. 1, *g*). The bract, unlike that of *Salix*, is lobed or lacerate (text fig. 1, *f, g*).

The two genera differ also in mode of pollination—the flowers of *Populus* being wind-pollinated (anemophilous) whilst those of *Salix* are predominantly entomophilous.

TAXONOMY OF SALIX

Andersson (15) groups the willows in three tribes which are in turn subdivided into sections and groups. The tribes are separated chiefly on the basis of number of stamens present:

- A. Pleiandrae, stamens three to many.
- B. Diandrae, stamens two, filaments free.
- C. Synandrae, stamens two, filaments connate.

Investigation discloses the fact that on the basis of vascular supply the species fall naturally into two rather clear-cut divisions, since in anatomy the flowers of the synandrous group are closely like those of the diandrous group.

In the Pleiandrae 9 species were studied, both staminate and pistillate forms in 7 of these. In the Diandrae and Synandrae 26 species (in a considerable number both staminate and pistillate forms) were studied. It is believed that these 35 species are representative of the genus as a whole since they are well distributed through the various groups.

It may be noted that from the pleiandrous group four (*S. Bonplandiana*, *S. capensis*, *S. Humboldtiana*, and *S. safsaf*) out of the six species studied are tropical or subtropical forms, whereas the much larger diandrous group (with exception of *S. Daviesii* of doubtful affinity) is made up exclusively of arctic and north temperate species.

TYPE DESCRIPTIONS

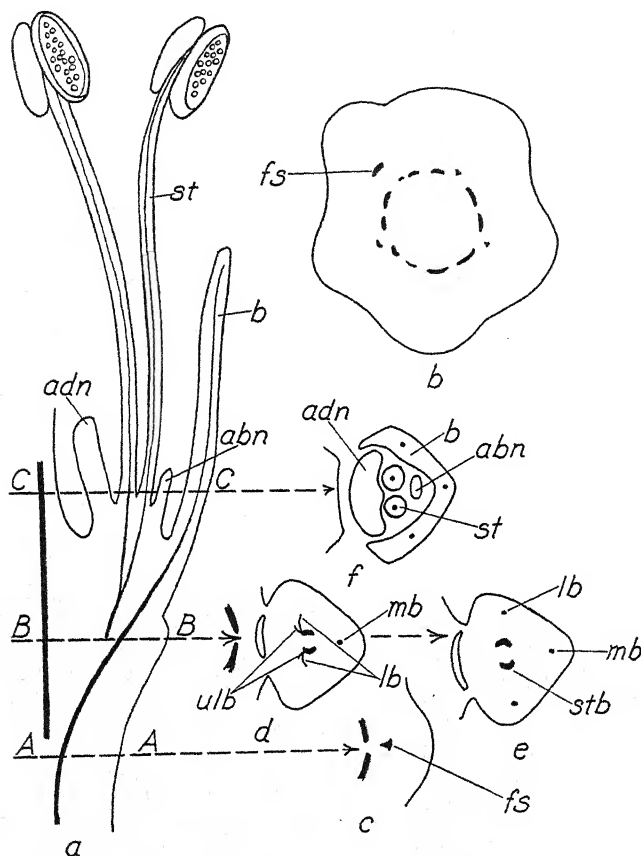
The origin and course of the vascular supply to the flowers of the diandrous group are so uniform that a detailed description of the supply to one staminate and one pistillate flower will suffice as a standard, and variations from this may be noted.

A description of the vascular supply to the staminate flower of *S. babylonica* may be used as illustrative of conditions typical of the diandrous group. Since the pistillate form of this species was not available, the vascular supply to the pistillate flower of the closely allied species, *S. alba*, is given as a type. Although Andersson places both these willows in the pleiandrous group, Gray includes them among the two-stamened forms. They are obviously transitional species but, since the vascular supply presents a combination of features characteristic of the diandrous willows as a whole, they are suitable for type descriptions.

I. DIANDROUS GROUP

Salix babylonica (text fig. 2). In this species the axial cylinder of the inflorescence is in the form of a dissected siphonostele with small gaps (text fig. 2, *b*). The vascular supply to the individual flower is first evident as a bundle which moves off from the axial cylinder in the form of a small wedge-shaped strand (text fig. 2, *c*) which gradually enlarges, fan-like. Meanwhile the bundle is migrating upward and outward through the cortex toward a ridge, triangular in cross-section, which has by this time become prominent upon the side of the catkin axis. While this bundle is still within the cortex, a delicate trace from the median portion of the fan-shaped strand passes out toward the apex of the ridge (text fig. 2, *d*, *e*). This bundle becomes the median trace of the bract, which is subsequently cut off. The gap caused by the outward passage of this trace breaks the vascular supply of the flower into two strands. Shortly after the passing out of this median trace another stronger bundle is given off from the adaxial edge of each of the two masses. These bundles become the lateral traces of the bract. From the basal ends of these lateral traces small strands (text fig. 2, *d*), which soon fade out, are occasionally pinched off. These are clearly stipule traces, as is evident in those species in which stipules are present. After the passing off of the traces to the bract, the two remaining masses of vascular tissue, now somewhat diminished in size, round up, move farther apart, and become definitely established as strands destined to supply the two stamens. One of these subsequently passes through the middle of each filament from base to apex (text fig. 2, *e*, *f*).

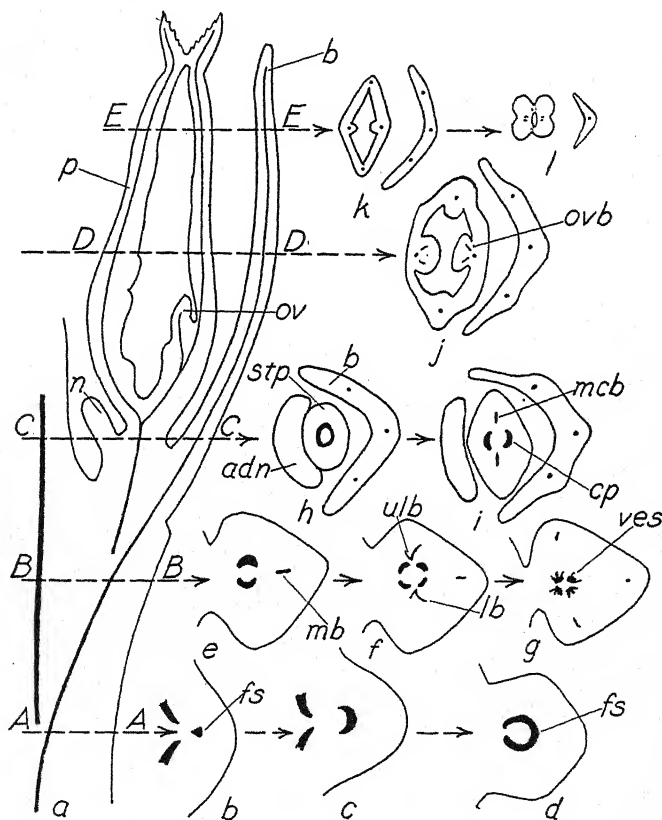
The flower itself may now be considered. The floral mass is next detached from the catkin-cortex, and, in this species, the bract is freed from the individual flower almost simultaneously with the severance of



TEXT FIG. 2. *S. babylonica* ♂: Diagrams illustrating origin, course, and distribution of the vascular supply to the staminate flower of a typical diandrous willow. *a*, section in median posterior-anterior plane; *adn*, adaxial nectary; *abn*, abaxial nectary; *b*, bract, *st*, stamen. *b*, transverse section through axis of catkin showing dissected siphonostele of the inflorescence and origin of floral strand (*fs*) from the stele. *c-f*, transverse sections of the flower at successively higher levels, *A-A*, *B-B*, *C-C*; *mb*, median vascular bundle to the bract; *lb*, lateral bundle to the bract; *ulb*, vestigial stipular bundle; *stb*, vascular bundle supplying stamen; *adn* and *abn*, adaxial and abaxial nectaries, respectively; *st*, stamen; *b*, bract.

the flower from the catkin. In the meantime the three bundles cut off as supply to the bract have moved outward into the bract and formed its three veins (text fig. 2, *f*). Following the severance of the bract, the flattened, somewhat ridged adaxial nectary (the larger of the two), as well as the abaxial gland, is freed from the two stamens. In this species there is no evidence whatever of a vascular supply to either nectary.

S. alba (text fig. 3). The origin of the vascular supply to the pistillate flower is identical with that of the staminate type-flower:—a wedge-shaped strand moves off from the axial cylinder and soon assumes a typical horse-shoe shape (text fig. 3, *a-d*), or, in some instances, the form of a complete siphonostele. As in *S. babylonica*, the median trace to the bract passes off somewhat in advance of the two lateral traces. The lateral traces in *S. alba*, however, come off, one each, from near the middle of the two masses into which the floral strand is broken by the passing out of the median trace (text fig. 3, *f*). Weak stipular strands which soon fade out were observed at the base of these lateral traces also. The small gaps of these lateral



TEXT FIG. 3. *Salix alba* ♀: Diagrams illustrating origin, course, and distribution of vascular supply to the pistillate flower of a typical diandrous willow. *a*, section in median posterior-anterior plane; *n*, nectary; *b*, bract; *p*, pistil; *ov*, ovule. *b-l*, transverse sections of developing flower at successively higher levels, A-A, B-B, C-C, etc.; *c* and *d*, sections from levels intervening between A-A and B-B; *f* and *g*, between levels B-B and C-C, etc.; *fs*, floral strand; *mb*, median vascular bundle to the bract; *ulb*, vestigial stipular bundle; *ves*, vestigial vascular bundle; *adn*, adaxial nectary; *stp*, stipe; *b*, bract; *mcb*, median carpellary bundle; *cp*, placental bundle; *ovb*, ovular bundle.

traces temporarily break the strand into four groups, but a readjustment of vascular tissue soon occurs and a siphonostele is formed (text fig. 3, *h*).

At this reconstruction period the vascular cylinder presents a ragged or frayed-out appearance because of the presence of remnants of radiating vascular strands which are strongly suggestive of vestigial bundles (text fig. 3, *g*). This condition, it will be observed, occurs above the passing off of the three traces to the bract and below the formation of the siphonostele which passes uninterruptedly through the center of the short stipe characteristic of the pistillate flower to the base of the ovary. At the flattened base of the ovary the siphonostele opens out and is finally broken up into four strands (text fig. 3, *i*), the two smaller of which pass out horizontally at either end. These are the two median or dorsal carpellary bundles forming the midribs of the carpels. The two considerably larger bundles pass to the placentae. Each of these two bundles represents two fused lateral carpellary traces, one from each carpel, the fusion resulting from proximity. Branches from these bundles supply the anatropous ovules. All four bundles continue up into the stigma.

The floral mass is severed from the catkin shortly after the formation of the siphonostele, but the bract is not freed from the individual flower until shortly afterward. Lastly, the single nectary is freed from the stalk of the ovary (text fig. 3, *h*, *i*). No indications of a vascular supply to the nectary were noted.

Variations in the Diandrous Group

Vascular supply to the bract. The origin, course, and distribution of the vascular supply to the bract of the diandrous willows are mostly typical, but occasionally variations occur which are suggestive of transitional phases between the mode now obtaining in the more highly evolved species of the diandrous group and that commonly found in many of the pleiandrous forms. Two or more variations not infrequently appear in the bract of flowers in the same ament. This was found to be the case in the staminate flowers of *S. amplifolia*, *S. arctica*, and *S. saximontana*. Two variations noted in *S. amplifolia* are as follows: (*a*) The median trace passes out in the usual way and becomes established in the apex of the triangular ridge on the side of the catkin; one lateral trace also arises typically, but the other originates as a branch which comes off at right angles from the median trace; (*b*) the second variation, the significance of which is discussed on page 318, is likewise concerned with the lateral traces both of which in this instance originate as horizontal branches of the more constant median trace.

In *S. arctica* two traces sometimes leave the median portion of the floral strand simultaneously and establish themselves at either side of the apex of the floral ridge. These traces, which leave a single gap, apparently constitute the only veins of the bract.

The lateral traces to the bract of staminate flowers are usually given off from the adaxial ends of the two vascular masses into which the floral strand is divided by the passing out of the median trace (text fig. 2, *d*). However, in several species (*S. amplifolia*, *S. arctica*, *S. cordata*, *S. rotundifolia*) neither this mode nor that followed almost invariably by the pistillate forms is found to the exclusion of the other, but both methods are found in staminate flowers of the same species.

From the basal ends of each lateral trace to the bract a delicate stipular bundle is commonly pinched off. These persist for some distance in *S. purpurea* ♂, *S. tristis* ♂, *S. vestita* ♂, *S. reticulata* ♀, etc.

Among other minor variations are the passing off of the two lateral traces in advance of the usually more precocious median trace, and the passing off of the lateral traces one in advance of the other.

Vestigial bundles. After the passing out of the traces to the bract, the remaining vascular tissue, as described in the type form, *S. alba*, commonly presents a ragged appearance due to the presence of stubby ends and fragments of bundles, of varying degrees of lignification, which usually pass off from the adaxial side of the vascular complex in the direction of the as yet undifferentiated adaxial nectary, but which also not infrequently radiate from the entire periphery of the stele. These vascular remnants, although quite definite, usually disappear a short distance from their source of origin; frequently, however, one or occasionally two (*S. alaxensis* ♀, *S. uva-ursi* ♀) whorls of isolated masses of small unligified but angular cells more or less completely surround the stele. These groups of cells, which soon fade out upward, undoubtedly represent the distal ends of the ragged strands which radiate from the periphery of the stele, the connections between which have entirely disappeared during the process of reduction of floral parts.

Direct connections between these adaxially projecting or radiating strands and the one to three vascular bundles or vestiges of bundles appearing in the nectary or nectaries of many species (*S. alaxensis* ♀, *S. amygdaloides* ♀, *S. cordata* ♀, *S. orbicularis* ♀, *S. reticulata* ♂, *S. tenuis* ♂ and ♀, *S. tristis* ♀, *S. uva-ursi* ♀, *S. vestita* ♀) are now with few exceptions (*S. alaxensis* ♀, *S. Bonplandiana* ♀) lost, but the gradual fading out of a former functional vascular supply, both within and without the nectary, is quite evident to-day. In the light, therefore, of processes still going on, the past history of these vascular remnants and areas of unligified tissue may be confidently read. In some species the vascular elements of these radiating or isolated strands are strongly lignified; in others exceedingly delicate bundles are found in which, however, spiral vessels may still be made out; and in still others only phantom-like areas of what may be termed "suggestive tissue," occurring in the same relative positions, serve to mark the former site of a now vanished vascular supply.

Nectary

In addition to the vestigial vascular supply persisting in the nectaries of various species of *Salix*, the lobing and relative position of these organs present some interesting and suggestive features. In the staminate flowers of *S. argyrocarpa* and *S. babylonica* the nectary, which completely surrounds the basal part of the stamens, is differentiated at a higher level into adaxial and abaxial glands, the latter of which breaks up near the distal end into from two to five lobes which alternate more or less definitely with the stamens. In *S. vestita* ♂, and in *S. orbicularis* ♀, both adaxial and abaxial glands break up into lobes, frequently three each, the position of which in *S. vestita* is highly suggestive of the alternate arrangement of perianth parts.

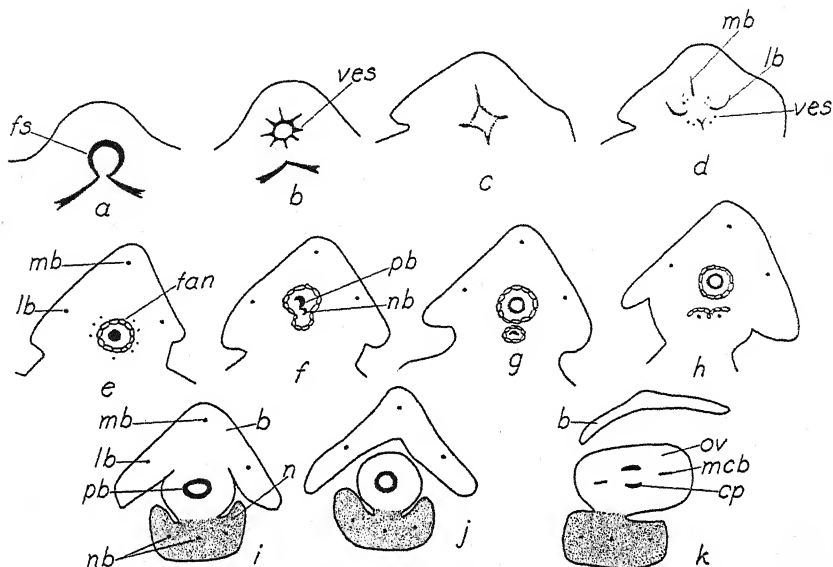
The peculiarly fused nectary with its vestigial vascular bundles is a striking feature of the staminate flower of *S. reticulata*. A short distance above the base of the stamens, however, the gland separates into the usual adaxial and abaxial nectaries. The former, and also occasionally the latter, becomes tri-lobed or tri-parted at its distal end. Vestigial vascular elements were observed in the three lobes of the adaxial gland. The abaxial nectary as a rule lacked evidences of a former vascular supply but in a few instances areas of "suggestive tissue" were apparent in the median lobe of this gland.

Significant Variations in Vascular Supply

The origin and distribution of the vascular supply to the floral parts of the two following species are described in considerable detail because of the obvious significance and importance of the vestigial bundles.

S. alaxensis ♀ (text fig. 4). The departure of the floral strand from the axial cylinder is not as a wedge-shaped segment as in *S. alba*, but as a ready-made stele, as it were. The strand when finally detached presents the appearance of a miniature stele which passes out through the cortex leaving a very small gap. As this stele migrates through the cortex isolated areas of suggestive tissue appear about its periphery. These it is believed are vestiges of preëxisting vascular strands, since they take exactly the same stain as do the thick-walled but apparently slightly lignified protoxylem vessels of the axial cylinder; their appearance is also very similar to that of other slightly lignified vascular tissue frequently observed, in which, however, spiral vessels are quite evident. Moreover, simultaneously with the appearance of this suggestive tissue, straggling remnants of vascular elements which radiate from the outer margin of the stele (text fig. 4, b) become somewhat conspicuous. These doubtless represent the proximal ends of bundles the distal parts of which are now only faintly indicated by the shadow-like presence of the suggestive tissue. At a slightly higher level the strand loses its ragged appearance, but shortly thereafter a spreading of the stele in four different directions at right angles to each other transforms the cylindrical strand into (in cross section) a diamond-shaped

mass (text fig. 4, c). From the apices of the abaxial and the two lateral angles, bundles pass off at practically the same level and establish themselves as the three bract traces. The strand which originates from the



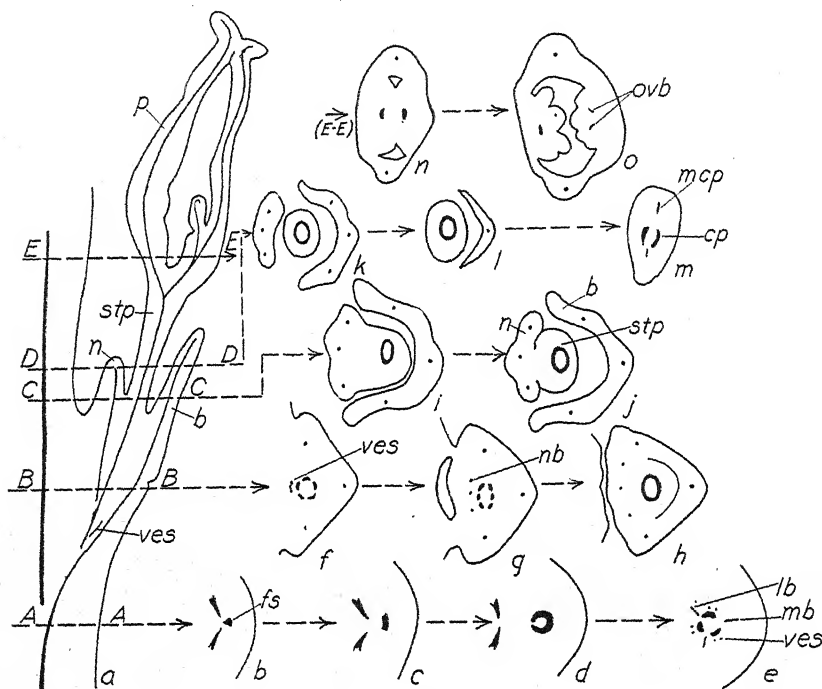
TEXT FIG. 4. *Salix alaxensis* ♀: Transverse sections of flower showing origin and course of vascular supply to the nectary. *fs*, floral strand; *ves*, vestigial vascular strands; *mb* and *lb*, vascular bundles to median and lateral portions of the bract; *tan*, sheath of tannin-filled cells; *pb*, vascular supply to the pistil; *nb*, vascular supply to the nectary; *b*, bract; *n*, nectary; *mcb*, median carpellary bundle; *cp*, placental bundle; *ov*, ovary.

apex of the fourth and inner angle passes off in the direction of the tissue of the, as yet, undifferentiated nectary and then disappears.

Suggestive tissue again makes its appearance about the margins of the strand (text fig. 4, *d*, *e*). By the time the traces to the bract have become definitely established in the floral ridge, the now depleted floral supply rounds up into a compact protostele-like strand which is sheathed by a cylinder of tannin-filled cells. Almost immediately thereafter there is pinched off from the adaxial side a strong bundle which is also invested with sheathing cells (text fig. 4, *e*, *f*, *g*). This large compact bundle moves out toward the tissue which is subsequently differentiated into the adaxial gland (the only nectary present in the pistillate flowers of this species), and ultimately establishes itself just posterior to the median ridge of the nectary where it persists as the vascular supply of this gland for a considerable distance before entirely fading out. In some instances this bundle subdivides into two or three branches which also supply the nectary (text fig. 4, *h*, *i*, *j*). Following the passing out and establishment of the bundle and its branches to the nectary, the remaining portion of the floral

strand again assumes the shape of a siphonostele in which form it passes through the short stalk of the pistil to the base of the capsule where it breaks up into the usual median carpellary and placental bundles.

S. cordata (text fig. 5). In this species the origin and structure of the vascular supply to the flower are, in grosser aspect, similar to that of *S. alba*, but in detail some suggestive variations occur.



TEXT FIG. 5. *Salix cordata* ♀: Diagrams showing, in addition to usual vascular supply, vestigial vascular bundles both previous to and subsequent to their appearance in the nectary. *a*, section in median posterior-anterior plane; *ves*, vestigial fibro-vascular bundle; *b*, bract; *n*, nectary; *stp*, stipe; *p*, pistil. *b-o*, transverse sections of flower at successively higher levels, *A-A*, *B-B*, *C-C*, etc.; *c*, *d*, *e*, sections between levels *A-A* and *B-B*; *g*, *h*, sections between levels *B-B* and *C-C*, etc.; *fs*, floral strand; *mb*, median bundle to bract; *lb*, lateral bundle to bract, *ves*, vestigial vascular bundles; *nb*, vascular bundle to nectary; *n*, nectary; *b*, bract; *stp*, stipe; *mcp*, median carpellary bundle; *cp*, placental bundle; *ovb*, vascular bundle to ovary.

As the floral strand segment passes slowly through the cortex it forms a stele from which the bract traces are cut off in the usual order. Almost simultaneously with the passing off of the median bract bundle and just previous to the passing off of the lateral ones, about six groups of bundles appear around the periphery of the floral stele (text fig. 5, *e*). The innermost (adaxial) groups of these delicate strands migrate toward each other and seem to fuse in a horizontal plane (text fig. 5, *f*). The outer ones soon

fade out. The three bract bundles have in the meantime become definitely established in a prominent ridge on the side of the catkin (text fig. 5, *f*, *g*). At this stage there is a second marked variation from conditions obtaining in *S. alba*, in that three bundles of extreme delicacy now appear in the tissue on the adaxial side of the diffuse floral stele (text fig. 5, *g*). These seem to be the continuation of the bundles or bundle-complex resulting from the fusion mentioned above. Shortly after the appearance of these three bundles the floral strand again rounds up into a siphonostele (text fig. 5, *h*), the bract is freed from the flower, and at about the same time the flower, bearing at its base the three bundles, is itself cut off from the catkin cortex. At the base of the flower the site of the nectary is first indicated by the appearance of three united lobes in which the three bundles are now visible (text fig. 5, *i*).

II. PLEIANDROUS GROUP

Although apparently working toward the goal already attained by the more highly evolved diandrous willows, the pleiandrous forms, more especially the staminate flowers, have not as yet settled upon any one anatomical plan which is followed sufficiently consistently to be designated a type. Therefore, after a general discussion of the bract, the staminate flowers are discussed by species and the pistillate together.

Vascular Supply to the Bract

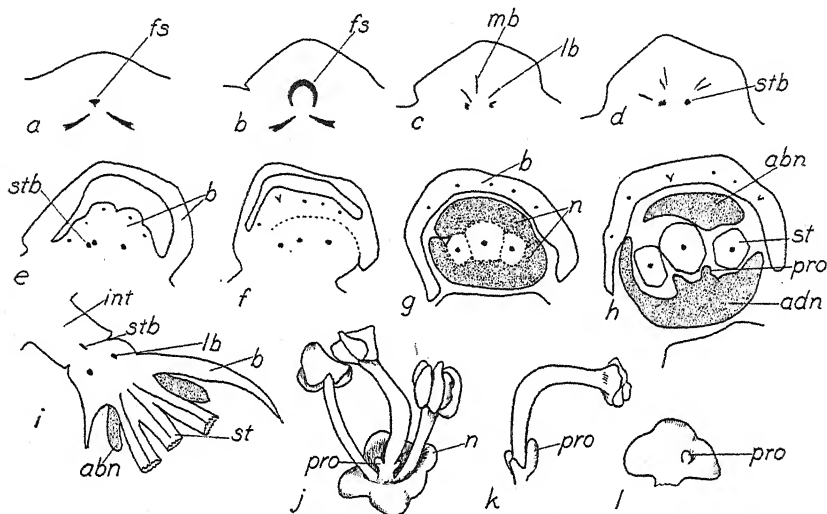
The predominant modes of origin of the vascular supply to the bract of these pleiandrous forms are so essentially alike that collective descriptions suffice. Likewise, as previously noted, similar modes of origin of vascular supply to the bract occur sporadically among the diandrous willows (*S. amplifolia*, *S. arctica*, *S. saximontana*).

In *S. amygdaloides*, ♂ and ♀, three strong traces, each of which bifurcates or trifurcates almost immediately, pass off (the median slightly in advance) from the median portion of the floral stele. The origin of the vascular supply to the bract of the staminate and pistillate flowers of *S. Bonplandiana* and *S. Humboldtiana* is practically identical. A single strong trace which bifurcates at once, or two adjacent traces of equal size, pass off medianly from the floral stele. In either case, a *single* gap is left in the stele. In *S. capensis* a single median trace from the center of the strand passes off first leaving the usual gap. The lateral traces originate either from the ends of the horseshoe, or from the proximal end of the median trace.

Staminate Flowers

S. amygdaloides (text fig. 6). Lack of stability in number of stamens and great variability in both form and number of nectaries characterize this species. Catkins consisting of flowers in which three stamens predominate are frequently found; others with flowers with four, five, or six stamens are, perhaps, even more common.

The floral strand moves off from the catkin cylinder in the form of the usual wedge-shaped segment (text fig. 6, *a*) which spreads open forming a horseshoe-shaped strand. The floral ridge in this species, as well as in the sterile flowers of all species of pleiandrous willows studied, is initiated as in the diandrous forms, but in the pleiandrous species this ridge is pro-



TEXT FIG. 6. *Salix amygdaloides* ♂: *a-h*, transverse sections of flower showing origin and course of vascular supply to floral parts of a pleiandrous willow with relatively few (three) stamens. *fs*, floral strand; *mb*, and *lb*, vascular supply to the bract; *stb*, vascular supply to stamen; *b*, bract; *n*, nectary; *adn* and *abn*, adaxial and abaxial nectaries; *pro*, nectariferous protuberance; *st*, stamen. *i*, longitudinal section of flower showing internode (*int*) at the summit of which floral parts are borne; *stb*, vascular supply to stamen; *lb*, vascular supply to bract; *b*, bract; *abn*, abaxial nectary; *st*, stamen. *j*, habit sketch of flower with bract removed showing microsporophylls encircled by nectary, and small protuberances at base of median stamen. *k*, median stamen of same flower; *pro*, protuberance. *l*, inner surface of adaxial nectary showing small nectariferous protuberance.

longed into a short, stout, lateral branch (text fig. 6, *i*) (apparently consisting of a single, incompletely suppressed internode), at the distal end of which the floral parts are borne. The horseshoe-shaped strand passes unbroken through this internode, at the summit of which a distribution of the vascular elements to bract and stamens takes place. Three bract traces depart leaving a single median gap. The two remaining masses of vascular tissue supply the stamens. In the three-stamened forms one supplies a lateral stamen, and the other divides to supply the remaining two (text fig. 6, *b-g*). Incidentally it may be noted that the relative position of these stamens, unlike that of the three-stamened forms pictured and described by Engler and Prantl and other authorities, is transversely linear, and not in the form of a triangle.

No vestigial strands were noted, although in the center of a few of the larger nectaries of the adaxial group minute areas which take a different stain from that of the surrounding parenchymatous tissue may, undoubtedly, be regarded as remains of a former vascular supply.

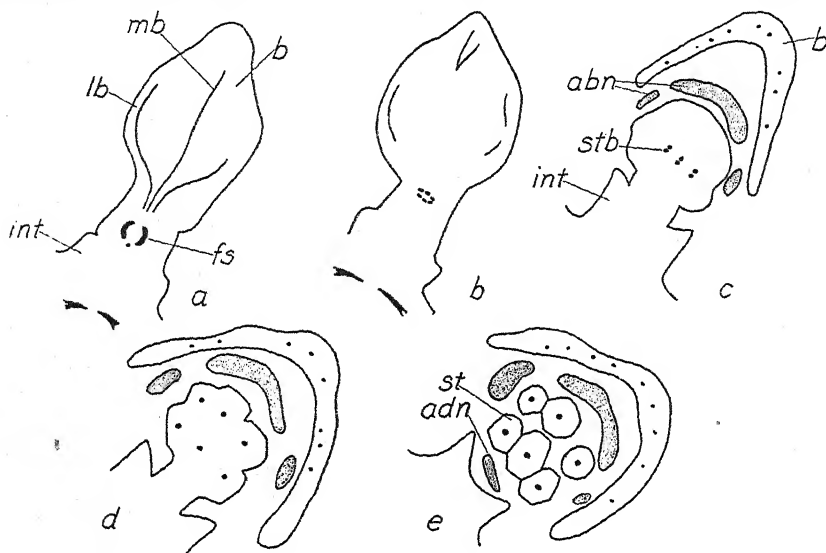
When more than three stamens are present in each flower the hemispherical masses remaining after the passing out of the traces to the bract are each resolved into two or three strands. There is some variation in the levels at which the strands are given off. That *S. amygdaloides* represents a species transitional between the Pleiandrae and the Diandrae is not only evinced by the inconstant number of its stamens and by the incomplete suppression of the internode or internodes intercalated between the flower and the axis of the inflorescence, but likewise by the exceeding plasticity of its nectaries, which surpass those of all other species examined in variety of form, number present per flower, and also even in their relative position. The basal part of the stamens of the three-stamened forms is usually encircled by a continuous, cup-like nectary which at a higher level is ordinarily differentiated into a varying number of lobes and glands of which the adaxial nectary is usually the largest. Small nectariferous protuberances in which there is not the slightest indication of a former vascular supply are occasionally found on the concave inner surface of the large flattened adaxial gland (text fig. 6, *l*) which curls about the stamens. These protuberances are parenchymatous in nature and take the same stain as the nectary proper, from the basal part of which they have their origin. Near the base of the filament of the median stamen in the three-stamened forms, two or three similar protuberances are also commonly found (text fig. 6, *j*, *k*).

S. Bonplandiana. In this species the actual insertion of the individual flower upon the axis of the catkin itself is far below its apparent emergence, since the vascular tissue which supplies the floral parts originates from the axial cylinder as a segment which quickly assumes the form of a protostele or of a compact siphonostele far below the first indication of the floral ridge. This compact stele moves very slowly outward through the cortex of the catkin to the floral ridge, which is later prolonged into a reduced internode similar to that described in *S. amygdaloides*. The floral stele passes as a single strand through this reduced, somewhat cup-like internode into a thickened, compact pedicel or receptacle which has the appearance of being telescoped. At the summit of this "pedicel" the strand is resolved into vascular elements which supply the various floral organs.

The stamens are surrounded by an indefinite number of nectaries which are variously lobed and divided. A striking feature, the significance of which will be discussed later, of these so-called nectaries, is that they do not take the usual stain, and are very clearly *not nectariferous* in nature. The adaxial gland is large and frequently broad and petaloid in appearance.

S. capensis (text fig. 7). The floral strand passes off from the catkin

cylinder in the form of the usual wedge-shaped bundle which rapidly spreads open into the typical horseshoe-shaped strand. This strand passes to the floral ridge, which becomes a concave (in section two-lobed), receptacular internode into which the basal part of the pedicel is sunken

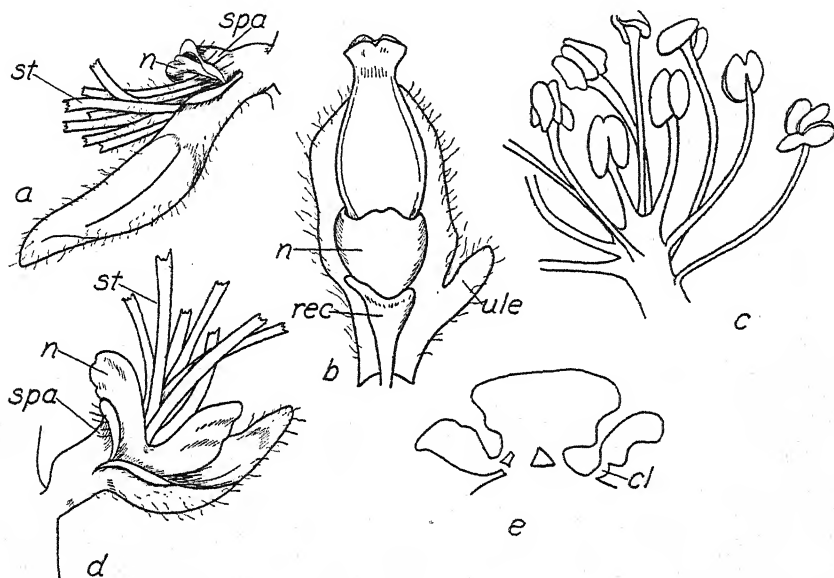


TEXT FIG. 7. *Salix capensis* ♂: Diagrams of flower showing origin and course of vascular supply to floral parts of a pleiandrous willow with many (six) stamens. *a, b*, in somewhat longitudinal plane; *int*, internode; *fs*, floral strand; *b*, bract; *lb* and *mb*, vascular supply to bract. *c, d, e*, transverse sections; *int*, internode; *stb*, vascular supply to stamen; *b*, bract; *abn*, abaxial nectary; *adn*, adaxial nectary; *st*, stamen.

(text fig. 7, *a-d*). After traces to the bract have passed off, the vascular tissue remaining in the pedicel temporarily masses together (text fig. 7, *b*), but very shortly thereafter is resolved into three strong bundles each of which subsequently forks into two equal branches (text fig. 7, *c, d*). The six bundles resulting from this forking ultimately become the vascular supply of the normally six stamens. The freeing (at practically the same level) of the stamens which soon follows, results in the formation of a whorl of microsporophylls arranged in two transversely placed parallel rows of three stamens each, the abaxial members of which are opposite those of the adaxial row (text fig. 7, *e*).

The most striking and significant feature of this species, however, is that the tissues of the well-developed so-called "nectaries" are not at all nectariferous in character, but take a stain like that of the pedicel or other adjacent tissues. The abaxial group of nectaries, in contrast with conditions prevalent in most other species of *Salix*, is more strongly developed than the adaxial gland, which, in some flowers, is entirely lacking. These abaxial nectaries usually appear in groups of threes of which the well-developed middle gland is frequently broad and petal-like.

S. Humboldtiana. The vascular supply to the staminate flower of this South American willow originates, like the supply to the pistillate flower of the arctic species, *S. alaxensis*, as a miniature, complete stele which is thrust out from the axial cylinder in the form of a rounded loop. This tiny stele makes a relatively long journey through the cortex of the catkin before establishing itself in the floral promontory. As in other pleiandrous willows, a short internode is traversed before the floral stele is broken up. The behavior of the vascular supply during the remainder of its course is closely similar to that of *S. capensis*.



TEXT FIG. 8. *Salix safsaf* ♂ and ♀: Habit sketches of staminate and pistillate flowers. *a*, scale and basal part of staminate flower showing fusion of the former with spathe-like structure, *spa*; *n*, nectary; *st*, stamens. *b*, pistillate flower with basal part of the scale freed from the receptacle showing well-developed, free stipule; *n*, nectary; *rec*, receptacle; *ule*, stipule. *c*, staminate flower with scale removed, showing spiral arrangement of microsporophylls. *d*, staminate flower with bilabiate nectary (*n*). *e*, petaloid nectary from staminate flower showing claw-like (*cl*) attachments.

S. safsaf (text fig. 8). The mode of origin of the vascular supply to the staminate flower of this willow, native of northern Africa, is like that of *S. Humboldtiana*. The internode, or internode and pedicel, in this species, however, is enveloped by a peculiar spathe-like structure, into the spreading distal ends of which the floral parts appear to be inserted (text fig. 8, *a*). This superficial resemblance to a diminutive spathe is further enhanced by the fusion of the basal part of the conspicuous concave bract with the sides of this structure which at the distal end of its adaxial side terminates in a free, revolute margin, the outer surface of which is sparsely beset with

long, conspicuous hairs (text fig. 8, *a, d*). In the pistillate flowers of this species a well developed, free stipule is occasionally found at the base of the bract (text fig. 8, *b*), in the same relative position as the "spathe" in the staminate flower. This suggests that the latter is probably homologous with the fused stipules which, together with the bract, may be here interpreted as a perfoliate leaf.

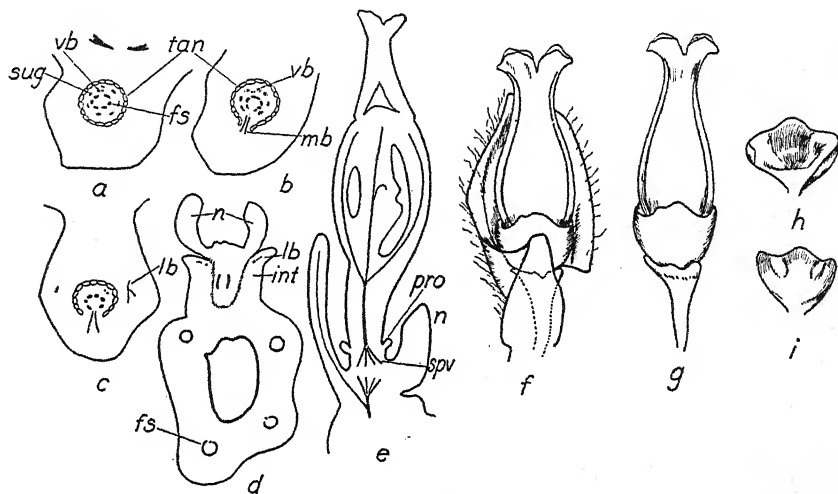
The vascular supply to the flower is initiated as a loop of tissue which is converted into a more or less perfect siphonostele by the time it is detached from the axial cylinder. The course of the strand through the cortex of the catkin and into the pedicel shows no unusual features. After traces to the bract have been given off, the two remaining vascular masses immediately bifurcate or trifurcate, and each division may in turn branch, depending upon the number of stamens (which range from seven to fourteen) to be supplied. The stamens are freed at different levels and are spirally arranged upon a stalky, trunk-like pedicel (text fig. 8, *c*).

The prominent nectaries of the staminate flower of this species are characterized by great diversity of size, form, number of parts, etc. They do not take the nectary stain as a whole, but small isolated areas may be somewhat nectariferous in nature. In some flowers the fusion of the basal parts of the nectaries, which completely encircle the lower part of the filaments, results in the production of a diminutive bilabiate structure, surprisingly suggestive of a miniature labiate flower (text fig. 8, *d*); in others little or no fusion occurs. All gradations may be found between these two extremes. The adaxial group consisting of one nectary, or more frequently of two or three, is inserted in the throat of the spathe-like envelope which surrounds the base of the stamens. The abaxial group, usually three in number, is intimately associated with the scale. Nectaries of this group are larger and better developed than those of the adaxial set. In this group the middle gland is frequently broad and extremely petal-like at its free end, but contracts abruptly at the basal end into one or two short claws which serve as means of attachment. The glands at either side are more slender and are also attached by means of claws (text fig. 8, *e*). No evidences of vascular supply were noted in any of the nectaries, possibly due to the fact that cells with dark staining content may have obscured vestigial elements.

Pistillate Flowers

The pistillate flowers of the pleiandrous willows do not differ materially from those of the diandrous group so far as origin of the floral stele and ultimate distribution of the vascular supply to the capsule are concerned, but in the intervening region there are certain significant differences which should be recorded. Most noteworthy among these differences is the invariable presence, in the species studied, of a short internode as described in the staminate flowers of *S. amygdaloides*. However, in these pistillate

flowers (*S. amygdaloides*, *S. Bonplandiana*, *S. Humboldtiana*, *S. nigra*, *S. safsaf*) the internode is not so obvious as in the staminate forms of the same species; it has evidently undergone still further suppression in the fertile flowers. In *S. Humboldtiana* the compressed internode seemingly functions as a sort of receptacular cup for the floral structures sunken within it (text fig. 9, *d*).



TEXT FIG. 9. Diagrams and sketches of pistillate flowers of pleiandrous willows. *S. Humboldtiana* (a-e): a, b, c, transverse sections of flower showing, in addition to usual vascular supply, presence of "suggestive tissue" (*sug*), and vestigial bundles (*vb*); *tan*, tannin-filled cells; *mb*, duplicate median bundles to bract; *lb*, lateral bundle to bract; d, transverse section of axis of inflorescence showing internode (*int*) into which the floral parts appear to be inserted; *n*, nectary; *lb*, lateral bundle to bract; *fs*, floral strand; e, longitudinal section showing spindle-shaped arrangement of vascular strands (*spv*) above which are small nectariferous protuberances; *n*, nectary. *S. safsaf* (f-i): f showing fusion of basal part of scale and stipules; g, scale removed to show cup-shaped nectary and obliquely lengthened receptacle; h, i, nectary flattened out showing inner (*h*), and outer (*i*) surfaces.

After the passing out of traces to the bract and during the period of readjustment of the remaining vascular tissue of the stele, remnants of two or three adaxially projecting strands from the periphery of the stele pass off in the direction of the nectary, but usually fade out before establishing direct connections with the bundle or remnants of bundles within the nectary itself.

In *S. amygdaloides* a delicate bundle persists in the median portion of the nectary in some flowers, and areas of suggestive tissue in the lateral extensions of these glands are indicative of the former presence of vascular elements in these also.

In *S. Bonplandiana*, as in *S. amygdaloides*, the more delicate of these adaxially projecting strands fade out before reaching the nectary, but in

many flowers the course of one of these bundles may be followed from its origin within the floral stele to the median part of the single cup-like nectary, where it persists as a vigorous bundle for a considerable distance. Suggestive tissue in the lateral extensions of the nectary indicates that at least three bundles formerly supplied this organ. The nectary of the pistillate flower, like that of the staminate, is non-nectariferous in character.

In *S. Humboldtiana* the stele, as in the staminate flower, is invested with a sheath of tannin-filled cells between which and the stele itself islets of suggestive tissue, especially pronounced on the adaxial side of the strand, appear previous to the fragmentation of the strand (text fig. 9, *a, b*). The theory that these areas of suggestive tissue may be correctly interpreted as vestiges of vascular elements is substantiated by the fact that in some of the more conspicuous islets bundles subsequently make their appearance. After the traces to the bract have passed out the floral strand assumes, temporarily, the form of a spindle (text fig. 9, *e*), the only plausible explanation of which would seem to be that a whorl of traces originally came off from the stele at this point and passed out to structures which are no longer extant. Evidence of the correctness of this interpretation is supplied by certain significant external structures (reminiscent, unquestionably, of ancestral features) which were observed in a number of flowers of this species. These structures appear above the nectary in the form of minute, non-nectariferous outgrowths about the stalk of the ovary somewhat above the level of the equator of the spindle described above. These tiny structures are apparently vestigial stumps of a whorl of floral structures which formerly occupied a position between the nectary and the base of the stipitate ovary. It is possible that they represent lost stamens.

An interesting feature observed in the pistillate flower of *S. safsaf* is the unique pedicel or receptacle upon which the floral organs are borne. This structure is revealed when the enveloping "cup," formed by a fusion of the basal part of the bract with the fused, usually ligulate stipules, is torn open. In outline this receptacle is very suggestive of the obliquely lengthened disk of the flower of *Populus* (text fig. 9, *j, k*).

The nectaries of the fertile flowers of *S. Bonplandiana* and of *S. safsaf* are very much alike. In each species the cup-like nectary curls about the stipe, but does not fuse on the abaxial side. If the slightly two-notched nectary of *S. safsaf* be removed and spread open, a thinning out of tissue just below each notch is obvious. This doubtless is either reminiscent or else prescient of a three-lobed condition (text fig. 9, *h, i*).

CHEMICAL TREATMENTS FOR SHORTENING THE REST PERIOD OF POT-GROWN WOODY PLANTS

F. E. DENNY AND ERNEST N. STANTON

(Received for publication February 18, 1928)¹

When woody plants such as lilac and flowering almond are grown in pots in the summer and are brought into the greenhouse in the succeeding fall in order to force them into bloom, it is found that a considerable period of time elapses before the buds open. Most species of woody plants exhibit this dormant period, the length of which varies greatly with the kind of plant and with the conditions (especially temperature) to which the plant is exposed just previous to transference to the warm house.

Johannsen (5) showed that treatment with chemical vapors such as ether and chloroform would break this dormant period and permit prompt growth of buds. Howard (4) in a series of experiments confirmed and extended this work and applied the treatments to a number of species. Stuart (6) showed that not only ether and chloroform but a number of other chemicals including ethyl bromid, ethyl iodid, carbon tetrachlorid, etc., could be successfully used. Our work extends these observations and adds to the list a number of other chemicals not previously used, two of which give promise of being specially valuable for practical work.

Because of the success that was obtained with certain chemical vapors in the treatment of dormant potato tubers (Denny 2, 3), tests of these chemicals and others of a similar nature were made. The experiments were carried out mainly with common lilac (*Syringa vulgaris* L.) and its variety "Charles X," flowering almond (*Prunus triloba* Lindl.), Bechtel's flowering crabapple (*Pyrus ioensis* Bailey), *Azalea nudiflora* L., *Deutzia gracilis* Sieb. and Zucc., and snowball (*Viburnum tomentosum* Thunb.).

Favorable results were obtained with all the species tested except snowball. The vapors of such chemicals as ethylene chlorhydrin, propylene chlorhydrin, ethylene dichlorid, vinyl chlorid, furfural, carbon tetrachlorid, acetylene tetrachlorid, ethyl bromid, and ethyl iodid forced early development of both leaf and flower buds. The gain in the time of budding varied from about two weeks in the case of flowering almond to as much as two months in the case of crabapple.

This paper describes the methods that were used in applying the chemicals, the concentrations and times of exposures that were found to be most favorable, and the responses that were given by the different species.

¹ Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

METHODS

Preparation of Plants for Treatments

Woody plants were obtained from nurseries in the spring of 1927. The lilacs, crabapples, flowering almonds, and snowballs were about three to four feet high, and the deutzias were about two feet high. The plants were potted into 12-inch clay pots and were buried, pot and all, in the soil, the top of the pot being at the surface of the soil. The plants made only a fair amount of growth during the summer of 1927, their growth being seriously interfered with by attacks of aphids. The flowering almonds formed abundant flower buds, and the deutzias formed a medium number of flower buds, but flower-bud development was sparing or absent in the case of the lilacs and crabapples. After the first frost in the fall (about November 8) the potted plants were removed from the soil and were stored in a basement room at a temperature of about 18° C. The plants, being stored indoors until treatments began, were not exposed to those low temperatures of outdoor conditions which are favorable for the loss of dormancy. Hence the plants remained in their rest period over a longer time, thus affording better opportunities for studying the effects of chemical treatment. The treatments began November 14 and continued until December 24.

Method of Exposing Plants to Chemical Vapors

Two types of containers were used: (1) A square galvanized iron box, capacity 4,200 liters, with a tight fitting door; an electric fan (of non-sparking type to avoid danger of an explosion) was installed in the box and at the beginning of each treatment the fan was turned on for about an hour in order to distribute the vapors within the enclosure. (2) Several galvanized iron cylinders, 800 liters capacity, equipped with hoisting apparatus; the galvanized iron base was made with a circular slot into which the bottom of the cylinder fitted, the seal being made with moist sand; the chemicals were introduced at the top into a shallow pan, evaporation being spontaneous and not aided by air currents. The temperature during the treatments was about 18° C.

Procedure After Plants Were Treated

The treated plants were placed in a greenhouse at a temperature of about 70° F. and were examined from day to day for bud development. When the buds opened the pots were fertilized with manure in order to supply the developing buds with proper nutrient supply. In each case the check plants were placed in the greenhouse at the same time, and were fertilized in the same way, whether or not the buds showed any signs of development.

RESULTS

Flowering Almonds (*Prunus triloba* Lindl.)

Untreated plants of this species began to show development of buds on December 23, and the first blooms opened December 28. The development of blooms in the check plants was irregular. *Prunus* plants treated with vapors of ethylene dichlorid, using 10 cc. of the liquid chemical per 100 liters of air space in the container, came into full bloom on December 13. This result is shown photographically in Plate XIX, *F*. The following treatments also gave early blooming of flowering almond: ethylene (one part of gas by volume to 100 parts of air for three days) (Pl. XIX, *H*); vinyl chlorid (one part of gas by volume to 100 parts of air for one day, and one part to 100 of air for three days); ethylene chlorhydrin,² 5 cc. for each 100 liters of air space inside the container for 24 hours (Pl. XIX, *G*). In addition to blooming sooner than the checks, the treated plants bloomed more uniformly, *i.e.* the various flower buds on a plant opened nearly simultaneously.

Common Lilac (*Syringa vulgaris* L.)

Leaf-buds of common lilac, untreated checks, began to unfold January 10, 1928, the development, however, being very irregular, as is shown in Plate XIX, *I*. The checks in fact were not in full leaf at the time the experiments with this species were discontinued on January 23. Common lilac plants treated November 29, 1927, with ethylene chlorhydrin using 5 to 10 cc. to each 100 liters of air space for 24 hours started development of buds in about 10 days after treatment and the plants were in full leaf in about 20 days; ethylene, one part of gas by volume to 1,000 parts of air for three days, caused the swelling of buds in 10 days and, while the subsequent opening of buds was somewhat irregular, a fair amount of leaf growth was attained in 30 days. Ethylene dichlorid, 10 cc. of liquid per 100 liters of space for 24 hours and 2.5 cc. per 100 liters of space for 48 hours, gave good results (Pl. XIX, *J*), as did also the following: propylene chlorhydrin, 8 cc. of the 40-percent solution per 100 liters of space for 48 hours; acetylene tetrachlorid, 0.5 cc. of liquid per 100 liters of space for 24 hours; furfural, 1 cc. of the commercial liquid per 100 liters of air space for 24 hours; vinyl chlorid, one part by volume of the gas to 250–1,000 volumes of air for three days (Pl. XIX, *K*); and carbon tetrachlorid, 2 cc. of the liquid per 100 liters of space for 24 hours.

Lilac (Variety "Charles X")

The buds of the untreated shrubs did not start to open until January 18, 1928, and the development of buds then occurred irregularly; none of the checks was in full leaf at the end of the experiment. Favorable results

² In this paper the ethylene chlorhydrin solution referred to is the 40-percent solution, the concentration ordinarily sold commercially; if the anhydrous 100-percent chemical is available it may be used by making allowance for the dilution.

were obtained with ethylene dichlorid using 10 cc. of the liquid chemical per 100 liters of space in the container for 24 hours. This result is shown in Plate XX, *P*. This plant was treated November 22, 1927, showed budding on December 2, was in full leaf December 12, and was photographed in full bloom December 23. Plate XX, *O*, shows the condition of the check plant on the same day. Other treatments that hastened the development of "Charles X" lilacs were: ethylene chlorhydrin, 5 cc. per 100 liters of space in the container for 24 hours (Pl. XX, *R*), and carbon tetrachlorid, 8 cc. per 100 liters of space for 24 hours (Pl. XX, *Q*).

Deutzia gracilis Sieb. and Zucc.

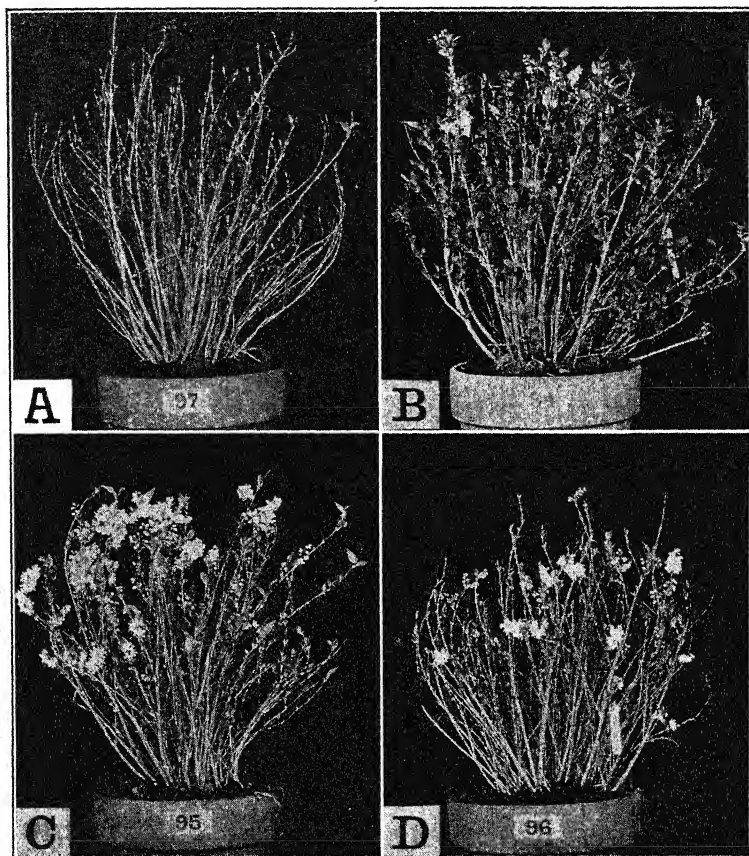
The buds on the untreated deutzias began to grow about December 15, but their rate of development thereafter was very slow and irregular. Some leaves and blooms were formed by the end of the experiment but none of the checks could be regarded as having produced a satisfactory development. The slowness of the rate of budding of the untreated plants may be seen by comparing the check plant in text figure 1, *A*, on December 28, with the check plant in Plate XIX, *L*, on January 16, 1928. *Deutzia gracilis* treated November 16 with ethylene chlorhydrin, 10 cc. per 100 liters of space for 24 hours, showed buds starting November 25 and was in full leaf December 3. This plant is shown in text figure 1, *B*; the photograph, however, illustrating the condition on December 28.

The object of text figure 1 is to show the effect of different concentrations of ethylene chlorhydrin upon the development of leaf buds as contrasted with flower buds. Thus, plant *B* was exposed to vapors of ethylene chlorhydrin using 10 cc. for each 100 liters of space for 24 hours; the concentration for plant *C* was one-fourth that for plant *B*; and for plant *D*, the concentration was one-fourth that for plant *C*. It is seen that the strongest concentration used caused the development of many leaves and few flowers; the weakest concentration, on the other hand, induced the development of flowers but no leaves; the medium concentration produced an intermediate development with respect to leaves and flowers. This effect was observed in another experiment with *Deutzia*, using ethylene dichlorid, but a similar result was not noted with any plant except *Deutzia*. Further experiments are planned to test the possibility of obtaining uniform results in controlling the development of leaf and flower buds in various species by varying the concentration of chemical vapor.

Other treatments that were successful with *Deutzia gracilis* were as follows: ethyl bromid, 5.5 cc. of the liquid chemical per 100 liters of air space for 24 hours (Pl. XIX, *N*); furfural, 12.5 cc. per 100 liters of space for 24 hours (Pl. XIX, *M*); dichlorethylene, 3 cc. per 100 liters of space for 24 hours; propylene chlorhydrin, 12.5 cc. of the 40-percent solution per 100 liters of space for 48 hours; acetaldehyde, 10 cc. per 100 liters of space for 24 hours; ethylene dichlorid, 10 cc. per 100 liters of space for 24 hours; vinyl chlorid 1 : 1,000 for three days.

Crabapple (Bechtel's Double-flowering, *Pyrus ioensis* Bailey)

This species was found to be very dormant under the conditions of our experiment. The untreated plants showed no signs of bud development on February 6, 1928. Treatment with ethylene chlorhydrin vapors, 2.5 to



TEXT FIG. 1. Effect of different concentrations of ethylene chlorhydrin upon the relative development of leaves and flowers of *Deutzia gracilis* Sieb. and Zucc. Plant A was the check plant receiving no treatment. Plant B was exposed for 24 hours to vapors of ethylene chlorhydrin, using 10 cc. of the 40-percent solution per 100 liters of space. The concentration for plant C was one-fourth of that for plant B, and the concentration for plant D was one-sixteenth of that for plant B. Note that plant B developed many leaves and few flowers, plant D produced flowers but no leaves, and plant C was intermediate with respect to these features. Plants treated November 16, photographed December 28.

10 cc. per 100 liters of space for 24 hours, caused the development of buds in about 10 days after treatment and gave plants with leaves fully opened in 15 days; the results are shown in Plate XX, T and U. Ethylene dichlorid, 10 cc. per 100 liters of space for 24 hours, induced early develop-

ment of buds and brought the plant into bloom on January 3, 1928. In the case of flowering crabapple, however, only a few plants grew sufficiently well during the summer of 1927 to produce flower buds. Consequently the effect of the treatments upon the blooming of flowering crabapple cannot be determined accurately from these experiments; however, the effect upon the growth of leaf buds was satisfactory. Other treatments that induced development of buds in advance of check plants were: vinyl chlorid, one part of gas to 250 parts of air for three days; propylene chlorhydrin, 12.5 cc. of the 40-percent solution per 100 liters of space for 48 hours (Pl. XX, V); and furfural, 12.5 cc. per liters of space for 24 hours.

Azalea nudiflora L.

Potted plants of this species were received from the Pierson Nursery, Tarrytown, N. Y., on December 23, 1927. Treatments were made December 23 and December 24 and the treated plants with their checks were placed in the greenhouse on December 24. The untreated plants developed flowers irregularly, the first flower opening on January 30, and the best blooming condition was reached on February 6. The treatments with ethylene chlorhydrin are shown in Plate XX, Y and Z. The buds of the plants exposed to vapors of ethylene chlorhydrin, using 6.7 cc. of the 40-percent solution per 100 liters of space for 24 hours, began to open on January 3, 1928 and this plant was in full bloom on January 17. Another plant (not shown in the photograph) was treated with one-third the concentration, that is, 2.2 cc. per 100 liters of space for 24 hours, and this plant bloomed about two days later than plant Y in Plate XX. Plant Z in Plate XX shows the result obtained with the weakest concentration of ethylene chlorhydrin used with *Azalea*, that is, 0.75 cc. per 100 liters of space for 24 hours. This plant bloomed on January 22. Thus, it is seen that the rate of development was influenced by the concentration of the chemical, the weaker concentrations inducing blooming somewhat later.

Snowball (*Viburnum tomentosum* Thunb.)

The various treatments that were successful in forcing early budding of the other species mentioned in this paper were not effective with *Viburnum tomentosum*. This species seems to require a different treatment. For the next series of experiments the plants will be given a preliminary exposure to low temperatures before the chemical treatments are started.

DISCUSSION

Condition of Plants Previous to Treatments

It was pointed out by Coville (1) that the duration of the dormant period of woody shrubs may be markedly shortened by exposing the plants to low temperatures for certain periods; but the plants in our experiments were not exposed to low temperatures at any time. Probably this explains

why the untreated shrubs retained their dormancy for such a long period, and why the growth which finally resulted was so irregular. The chemical treatments, however, overcame the dormancy even under these unfavorable conditions and permitted a simultaneous opening and uniform development of the buds. In future experiments it is planned to bring the potted plants in from the field at an earlier date, and to expose some of them to low temperatures for a few weeks before the chemical treatments are applied.

Behavior of Different Species

Of the species tested, the flowering almond plants (*Prunus triloba*) had the shortest dormant period, followed in order by *Deutzia gracilis*, common lilac (*Syringa vulgaris*), "Charles X" lilac, flowering crabapple (*Pyrus ioensis*), and snowball (*Viburnum tomentosum*). The *Azalea nudi-flora* plants were received some weeks after the other species and since they had been kept previously under a different set of conditions, it is not possible from the information in this experiment to compare the length of their rest period with that of the other species. After the rest period of the various species was broken the subsequent rate of growth appeared to be satisfactory and normal, except perhaps for the crabapple, in which case the growth was somewhat slow. It would be necessary to make comparisons with non-dormant plants under similar conditions in order to determine whether the treated plants were inferior in this respect. The blooms that were produced by treated plants developed well and appeared to be normal in all cases but one. A flowering almond plant produced flowers noticeably lighter in color than the others but it is not known whether this resulted from the chemical treatment or from a condition that existed in the plant before the treatment was applied.

Effectiveness of the Different Chemicals

The most favorable results were obtained with ethylene chlorhydrin and ethylene dichlorid. It will require further experiments to show which one of these two is the more effective. They are particularly promising, also, because of their ready availability in commercial quantities at a low price. The dichlorid is lower in price but an important advantage of the chlorhydrin lies in the entire absence of any danger of an explosion with its vapors at ordinary temperature. Among the chemicals that have not been used previously in experiments on hastening the budding of dormant woody plants but which gave good responses in these experiments are propylene chlorhydrin, vinyl chlorid, furfural, and acetylene tetrachlorid. Considerably less favorable results were obtained with iso-propyl formate, methyl salicylate, dichlorethylene, and trichlorethylene. Ethylene was tried in various concentrations and periods of treatment ranging from one part in 100 for three days to one part in 1,000 for one day. It was effective against the less dormant species such as *Prunus triloba* but did not induce

the development of the dormant buds of the more difficult species such as "Charles X" lilac and crabapple. A further disadvantage lies in the longer periods of exposure that are required, three to six days, for ethylene as compared with one day for certain of the other chemicals used. This same objection may also be made against vinyl chlorid, but the greater effectiveness of vinyl chlorid indicated the need of further experiments as to its usefulness.

The following chemicals employed by Stuart (6) in his experiments with potted woody plants were used in these experiments; chloroform, ethyl bromid, ethyl iodid, and carbon tetrachlorid. All of these induced budding considerably in advance of the untreated lots, ethyl bromid giving results somewhat better than the others. Of these four, chloroform gave the least favorable responses. Considerable injury was observed with the ethyl iodid treatments.

Future Experiments

It is proposed to continue these tests and to extend them to other species and to other chemicals. The variability of the plants has not been given proper consideration yet, and the question whether a group of plants of the same species can be forced into uniform development by a single treatment should have attention. Furthermore, the relation of the stage of dormancy of the plant to the concentration of the chemical and the duration of the exposure needed for good results should be established. The possibility of improving the methods by exposing the plants to a period of chilling previous to the application of the chemical treatments should be tested. Temperature effects during the period of treatment also need consideration.

SUMMARY

1. Woody plants, flowering size, were planted in the spring in 12-inch pots which were then sunk into the ground. The plants grew in these pots during the summer of 1927.
2. In the autumn of 1927 the potted plants were brought into the laboratory from the field, were placed in metal containers of 800 or 4,200 liters capacity, and were exposed to vapors of various chemicals for the purpose of breaking the rest period of the plants and inducing early development of buds and flowers.
3. The species used were lilac (*Syringa vulgaris* L.); flowering almond (*Prunus triloba* Kindl.); *Deutzia gracilis* Sieb. and Zucc.; crabapple (*Pyrus ioensis* Bailey); *Azalea nudiflora* L., and *Viburnum tomentosum* Thunb. These were brought into leaf or bloom by 24 to 48 hours treatment with the vapors of various chemicals except in the case of the *Viburnum*, which did not respond favorably. The gain in time required for the development of leaves or flowers by treated plants over the checks varied from two weeks in the case of *Prunus* to more than two months in the case of crabapple.

4. The most effective chemicals tried were ethylene dichlorid and ethylene chlorhydrin. The results were sufficiently favorable with propylene chlorhydrin, furfural, vinyl chlorid, and acetylene tetrachlorid to make further tests with them desirable.

5. In the case of *Deutzia gracilis* the relative development of leaves and flowers was modified by the concentration of the chemical used in the treatment.

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EXPLANATION OF PLATES

PLATE XIX

Top row shows results with flowering almond (*Prunus triloba* Lindl.).

Plant *E* was the check plant, not treated; photographed December 13. Plant *F* was exposed 24 hours to vapors of ethylene dichlorid using 10 cc. of the liquid chemical per 100 liters of space; treated November 22, photographed December 13. Plant *G* was exposed 24 hours to vapors of ethylene chlorhydrin, 5 cc. of the 40-percent solution per 100 liters of space; treated December 9, photographed December 28. Plant *H* was exposed three days to ethylene gas using one part of ethylene to 100 parts of air; treated November 14 to 17, photographed December 23. Note: Only plants *E* and *F* are strictly comparable photographically. Plants *G* and *H* were photographed at later dates, at times when the check plants were further developed, as described in the text.

Middle row shows results with common lilac (*Syringa vulgaris* L.).

Plant *I* was the check plant, not treated; photographed January 16. Plant *J* was exposed 48 hours to vapors of ethylene dichlorid, 2.5 cc. of the liquid chemical per 100 liters of space; treated December 10, photographed January 6. Plant *K* was exposed three days to vinyl chlorid gas, one part of vinyl chlorid to 1,000 parts of air; treated December 12, photographed January 12.

Bottom row shows results with *Deutzia gracilis* Sieb. and Zucc.

Plant *L* was the check plant not treated; photographed January 16. Plant *M* was exposed 24 hours to vapors of furfural, 12.5 cc. of the liquid chemical per 100 liters of space; treated December 6, photographed January 12. Plant *N* was exposed 24 hours to vapors of ethyl bromid, 5.5 cc. of the liquid chemical per 100 liters of space; treated December 1, photographed January 6.

PLATE XX

Top row shows results with "Charles X" lilac (*Syringa vulgaris* L.).

Plant *O* was the check plant, not treated; photographed January 16. Plant *P* was exposed 24 hours to vapors of ethylene dichlorid, 10 cc. of the liquid chemical per 100 liters

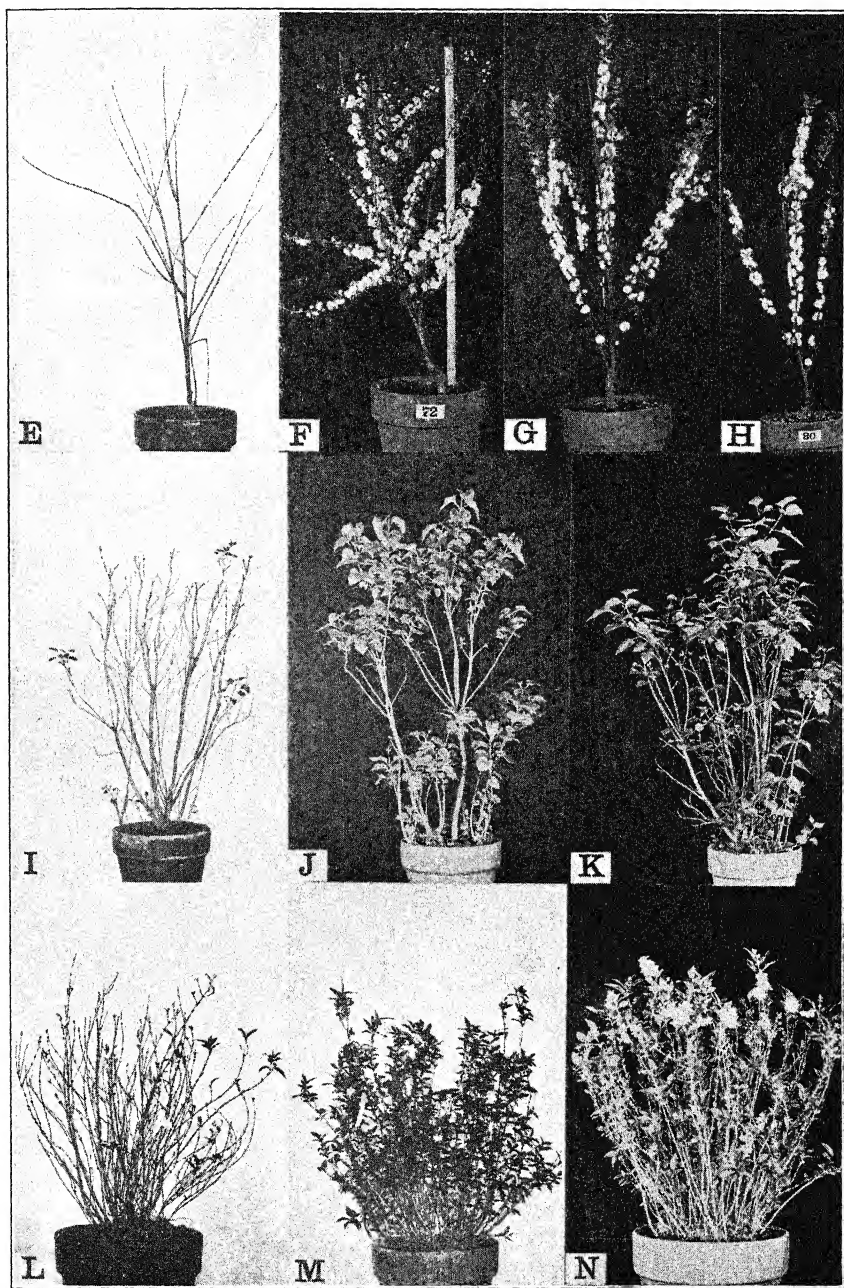
of space; treated November 22, photographed December 23. Plant *Q* was exposed 24 hours to vapors of carbon tetrachlorid, 8 cc. of the liquid chemical per 100 liters of space; treated December 14, photographed January 16. Plant *R* was exposed 24 hours to vapors of ethylene chlorhydrin, 5 cc. of the 40-percent solution per 100 liters of space; treated December 9, photographed January 16.

Middle row shows results with Bechtel's flowering crabapple (*Pyrus ioensis* Bailey).

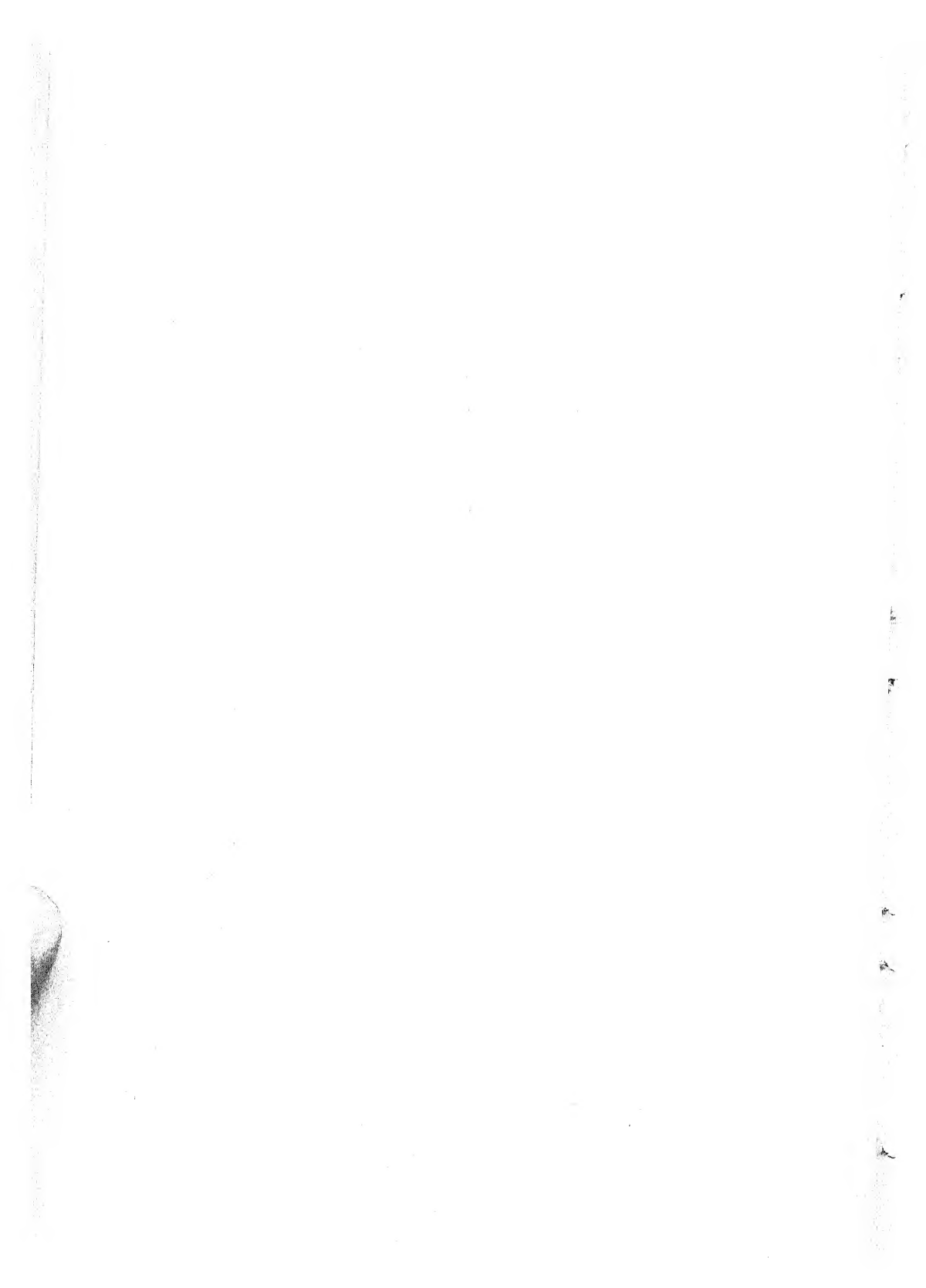
Plant *S* was the check plant, not treated; photographed January 16. Plant *T* was exposed 24 hours to vapors of ethylene chlorhydrin, 5 cc. of the 40-percent solution per 100 liters of space; treated December 9, photographed January 16. Plant *U* was exposed 24 hours to vapors of ethylene chlorhydrin, 2.5 cc. of the 40-percent solution per 100 liters of space; treated November 29, photographed January 16. Plant *V* was exposed 48 hours to vapors of propylene chlorhydrin, 12.5 cc. of the 40-percent solution per 100 liters of space; treated December 3, photographed January 16.

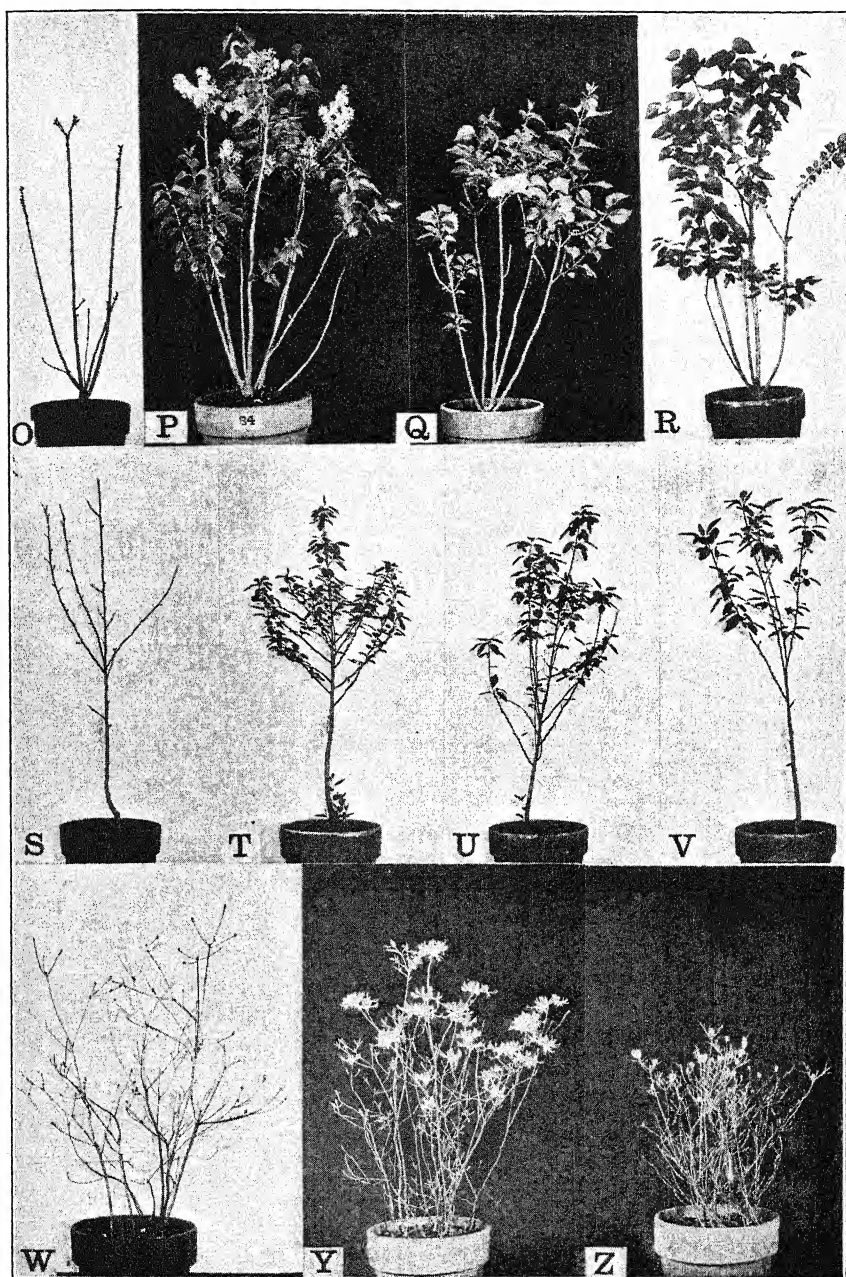
Bottom row shows the results with *Azalea nudiflora* L.

Plant *W* was the check plant, not treated; photographed January 17. Plant *Y* was exposed 24 hours to vapors of ethylene chlorhydrin, 6.7 cc. of the 40-percent solution per 100 liters of space; treated December 23, photographed January 17. Plant *Z* was exposed 24 hours to vapors of ethylene chlorhydrin 0.75 cc. of the 40-percent solution per 100 liters of space; treated December 23, photographed January 17.



DENNY AND STANTON: REST PERIOD





DENNY AND STANTON: REST PERIOD

LOCALIZATION OF RESPONSE OF WOODY TISSUES TO CHEMICAL TREATMENTS THAT BREAK THE REST PERIOD

F. E. DENNY AND ERNEST N. STANTON

(Received for publication February 18, 1928)¹

In the preceding paper (2) it was shown that dormant woody plants when grown in pots and exposed to the vapors of certain chemicals are forced into growth. In the present paper it is emphasized that individual twigs and buds upon the intact plant act independently, and that a single twig or a single bud will respond to this treatment if the selected twig or bud is allowed to come in contact with the vapor, while other twigs or buds are protected from exposure to it.

Lilac (*Syringa vulgaris* L.) proved to be a favorable plant for these experiments since the buds are opposite, and, at the tips of the twigs, are side by side in pairs. It was found possible to treat any one bud of such a pair and to induce the prompt growth of the treated bud without breaking the dormancy of the untreated bud located only a few millimeters away.

The buds or twigs that were started into growth in this way grew vigorously thereafter, showing that the roots and conductive tissues in the stem were not dormant, but were able to supply sap to the buds as soon as the buds were able to use it. The dormancy was not, therefore, systemic; only the buds themselves were dormant.

This view of the matter was first proposed by Howard (3, p. 24) because of the fact that in his experiments twigs without roots responded in a manner similar to rooted plants in pots, and because of the further observation that the buds were always the first organs to show a renewal of activity. By means of the experimental technique described below we obtained evidence that this view is correct.

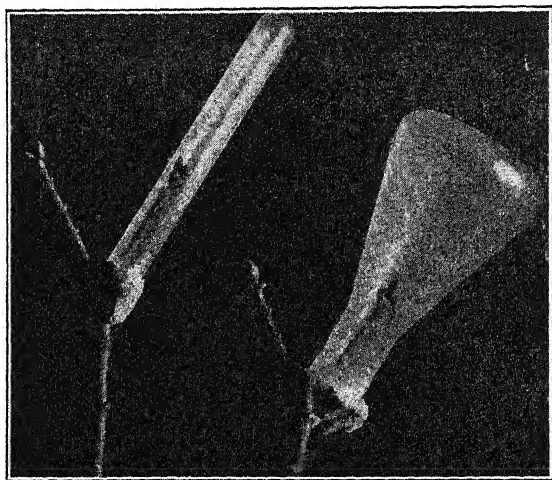
This paper emphasizes how narrowly the effect of the treatment may be localized in the tissue, and that, in fact, the influence of the vapor is upon the buds only.

TREATMENT OF INDIVIDUAL TWIGS ON THE SAME PLANT

In the preliminary experiments the tips of certain branches upon a plant were exposed to vapors of chemicals by the method shown in text figure 1. Into glass flasks and test tubes of sizes varying from 300 cc. to 28 cc. a drop of chemical was placed; the flask was then inverted over the twig and the opening between the twig base and the mouth of the tube

¹ Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

or flask was sealed with molding clay (plasteline). The check consisted in treating other twigs in exactly the same manner except that a drop of water was used instead of a drop of chemical.



TEXT FIG. 1. The method used in exposing individual twigs to vapors. A drop or two of the chemical was placed in the flask which was then inverted over the twig, the seal at the bottom of the tube or flask being made with molding clay (plasteline).

A typical case giving the results of such an experiment is shown in text figure 2. The treatments were made December 10, 1927, and the photograph was taken January 6, 1928. Twig *A* was exposed to vapors of ethylene chlorhydrin for 40 hours by putting one drop of 40-percent ethylene chlorhydrin in a 28-cc. test tube and inverting over the twig in the manner shown in text figure 1. The treatment for twig *B* was one drop of ethylene dichlorid; for twig *C*, one drop of ethyl iodid; for twig *D* one drop of acetaldehyde. For the check, twig *E*, one drop of water was placed in a 28-cc. test tube and the tube was inverted and sealed in a manner similar to that of the treated lots. The other buds upon the same plant may also be regarded as checks, since they received no chemical treatment. It will be seen that only the treated twigs started growth, and it will be noted that the growth was vigorous and healthy. The untreated twigs remained dormant. The activity that was induced in one twig by chemical treatment has not been communicated to neighboring twigs.

Another illustration of this effect is shown in text figure 3. Twig *A* was treated with vapors of ethylene chlorhydrin, using one drop of the 40-percent commercial solution in a 300-cc. flask, inverting the flask over the twig as shown in text figure 1, and letting stand 40 hours. Twig *B* received one drop of ethylene dichlorid in a 300-cc. flask, and twig *D* one drop of ethylene dichlorid in a 50-cc. flask. Twig *C* was the check, receiving

one drop of water in a 300-cc. flask. The treatment was given December 10, 1927, and the photograph was taken January 6, 1928. The interesting comparisons in text figure 3 are twig *A* with twig *E*, twig *B* with twig *F*, and twig *D* with twig *G*. Only the treated twigs started growth. Adjacent and comparable untreated twigs have remained dormant. Position upon the plant has not been an important factor, as is shown by twig *C* in text figure 2 and twig *D* in text figure 3. The chemical treatment has been the deciding factor for the growth of the buds on the twigs.



FIG. 2.

FIG. 3.

TEXT FIG. 2. Twig *A* was treated with ethylene chlorhydrin, twig *B* with ethylene dichlorid, twig *C* with ethyl iodid, twig *D* with acetaldehyde, and twig *E* with water. Other buds on the plant may be regarded as checks also, since they received no chemical treatment. TEXT FIG. 3. Twig *A* was treated with ethylene chlorhydrin, twigs *B* and *D* with ethylene dichlorid, and twig *C* with water. Other twigs on the plant, including twigs *E*, *F*, and *G*, were not treated. Compare twigs *A* and *E*, twigs *B* and *F*, and twigs *D* and *G*.

Text figures 2 and 3 show that the twigs upon the dormant plants act as individuals, and that any one twig may be aroused from its dormancy

while other twigs upon the same plant remain in the resting period. Thus, lilac responded to short exposures to vapors in much the same way as blueberry twigs to long periods of low temperatures as described by Coville (1).

TREATMENT OF INDIVIDUAL BUDS UPON THE SAME TWIG

The method of treating individual buds upon the same twig is shown in text figures 4 and 5. A disk of molding-clay (plasteline) is first placed

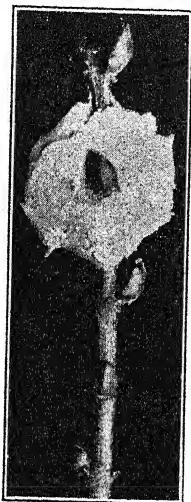


FIG. 4.

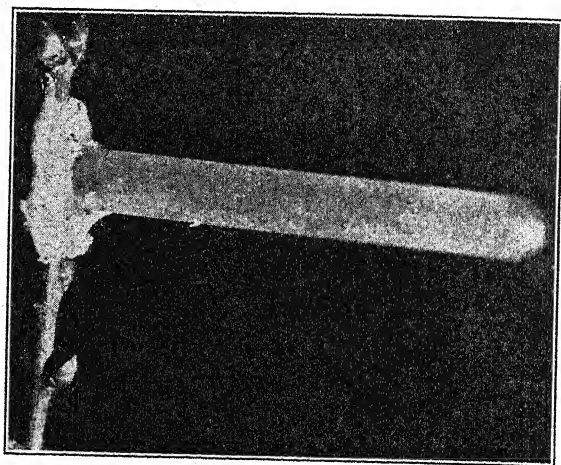


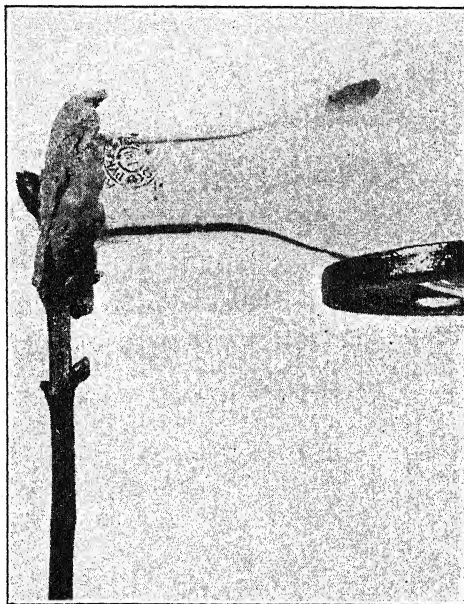
FIG. 5.

TEXT FIG. 4. Method used in treating individual buds. Molding clay (plasteline) was first placed around the selected bud in order that the chemical vapors could be applied to it without having them come in contact with the opposite bud on the other side of the twig.

TEXT FIG. 5. Method of applying chemical vapors to individual buds. A drop or two of chemical was placed in the tube, the mouth of which was then placed over the selected bud and the seal made with molding clay. The bud on the opposite side of the twig was not exposed to chemical vapors.

around the bud, as in text figure 4. The chemical is then dropped into the test tube or flask, and the mouth of the tube or flask is placed against the clay and the edges sealed. Only the bud within the tube is thus exposed to the vapors. The procedure for tip buds is shown in text figure 6. For checks upon this treatment the method is the same except that water instead of a chemical is used, and of course other buds upon the same twig or upon other twigs on the same plant may rightfully be regarded as checks, since they have not been exposed to chemical vapors. Separate experiments in which buds were partly or wholly encased in molding clay (plasteline) showed that contact of the buds with this material is not an important factor in these experiments.

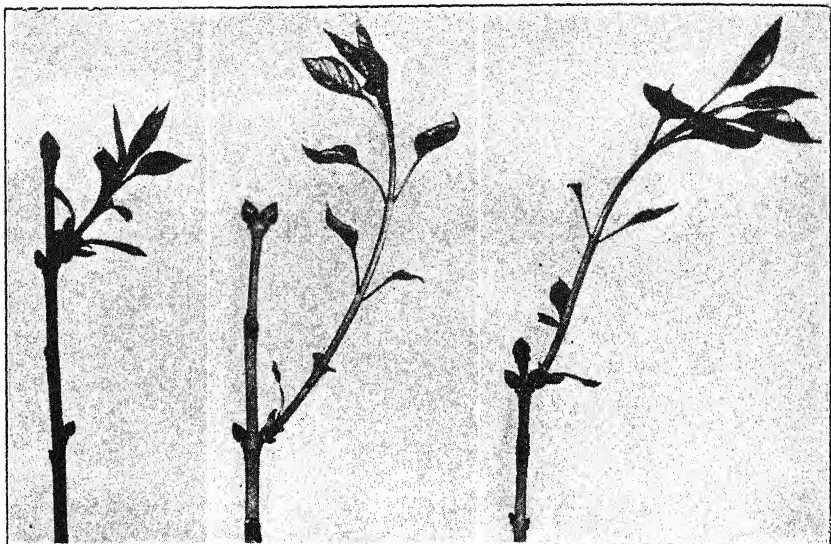
The results of tests with individual buds are shown in text figure 7. Single buds upon twigs of a potted plant of common lilac were treated with ethylene chlorhydrin vapors by the method described in the preceding paragraph. The chlorhydrin solution used was the 40-percent solution diluted 1 : 2 with water; one drop of this solution per 28-cc. test tube was used, and the time of exposure of the buds to the vapors was 24 hours. The treatments were made December 30, 1927, and the photographs were taken January 20, 1928. Only the treated buds started growth. The



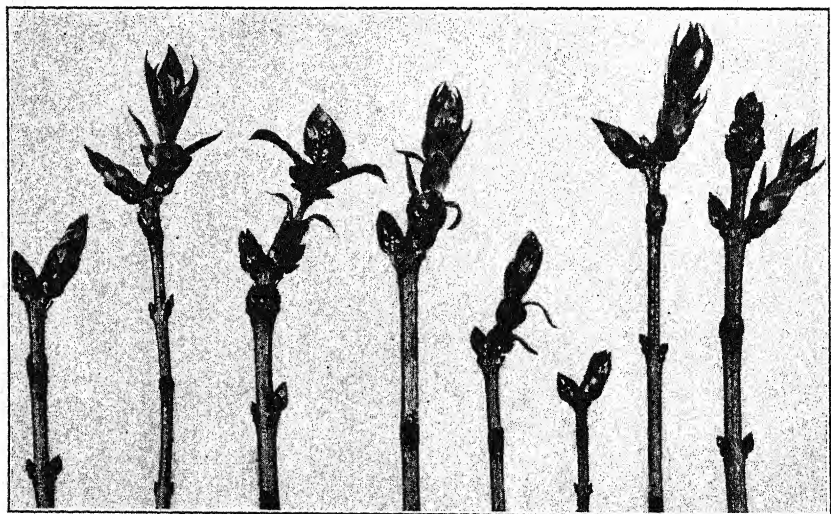
TEXT FIG. 6. Method of treating only one bud of the pair of buds at the tip of a twig of lilac. The molding clay layer protects the bud at the left from contact with the chemical vapor in the flask; the right-hand bud of the pair, however, is in contact with the vapors.

opposite bud, in each case located only a few millimeters away, received no treatment and remained dormant. None of the adjacent buds started into growth, showing that the effect of the vapors was narrowly localized in the bud.

The results of an experiment in which buds of the "Charles X" variety of lilac were used are shown in text figure 8. A single bud on each twig was treated with vapors of ethylene chlorhydrin, using the method shown in text figure 6. The ethylene chlorhydrin solution used was the commercial 40-percent solution diluted 1 : 4. In each case two drops of this solution were dropped into the tube or flask used for applying the vapor over the bud. In this series the size of flasks used varied from 10 cc. to 300 cc.



TEXT FIG. 7. Results of applying vapors to only one bud on each twig, using the method shown in text figures 4 and 5. The treated bud on each twig (common lilac) started into growth, but the other buds on the twigs received no chemical treatment and remained dormant. The bark, roots, and wood of the stem were not dormant, but could supply sap to the buds as soon as they could begin growth. Only the buds were dormant.



TEXT FIG. 8. Result of treating only the right-hand bud of the pair of buds at the tip of twigs of "Charles X" lilac, using the method shown in text figure 6. In the case of twig number 8 (counting from the left) the tip buds were not treated, but only the right-hand bud of the pair at the node below the tip. In each case the treated bud was brought out in advance of the untreated bud. For a discussion of such cases as buds number 2 and 7 (counting from the left) see text.

The treatments were applied to buds upon the intact plant; after the buds had made considerable growth the twigs were clipped from the plant and arranged for the photograph (shown in text figure 8) by placing the treated bud at the right and the untreated bud at the left in each case. The localization of the response in the individual bud is again shown in text figure 8. This also shows that the effect upon individual buds can be produced at will, and is not a rare occurrence that can be obtained only occasionally.

In some cases the opposite bud of the pair was also forced into growth. Buds 2 and 7 (counting from the left) in text figure 8 show this effect to a certain extent. Occasionally adjacent buds above and below the treated bud developed in advance of other buds on the same twig or plant. In such cases it cannot be stated whether the clay seal had channels in it that allowed the opposite bud to receive some vapor, or whether the factor or factors that induced the starting of growth in the treated bud were translocated in the stem across to the opposite bud. More experiments with reference to this feature of the work are desirable.

DISCUSSION

These results, showing that the effects of the vapor treatments in breaking dormancy are narrowly localized in the buds alone, are helpful to experimenters who are dealing with the causes of dormancy and with the effect of agents that break it. The experiments indicate that the evidence of changes accompanying the resumption of growth in resting plants should be sought in the buds and not in the adjacent tissue; that is, in taking tissue for examination, the bark, cambium, wood, and pith may, for the present at least, be regarded as of secondary importance. For example, in analyzing tissue to determine changes in enzym activity or chemical constituents, it would be undesirable to include in the sample entire twigs or tubers. It would appear likely that the inclusion of the extraneous tissue would obscure any changes that had taken place in the tissue of critical importance, the buds.

Moreover, it appears likely that only certain tissues *inside the buds* are important factors in the changes that are set up by the vapor treatments. Much work needs to be done in localizing these internal effects. Are there changes in the storage tissue within the bud that can be correlated with the resumption of cell division in the growing point? Or do the first detectible signs of change take place in the growing point itself?

SUMMARY

1. Individual twigs of dormant lilac plants growing in pots were exposed to vapors of ethylene chlorhydrin, ethylene dichlorid, and ethyl iodid, while similar twigs upon the same plant received no chemical treatment. The treated twigs started growth but the untreated twigs remained dormant.

The separate twigs on a given plant acted as units, and any one of them could be aroused from the rest period while adjacent twigs were inactive.

2. Likewise, individual buds upon twigs were exposed to vapors of ethylene chlorhydrin under suitable conditions, and were forced into growth by this treatment, while the bud upon the opposite side of the twig received no treatment and remained dormant. Of the two buds at the tips of twigs of lilac any one could be caused to develop new twigs without breaking the dormancy of the other bud of the pair.

3. Dormancy in the lilac is not systemic, that is, distributed throughout the plant. It is localized in the buds only. The roots, bark, and conductive tissues are not dormant, but are able to supply sap to the buds as soon as the buds are able to use it.

4. The experiments indicate that, in studying the dormant period, evidence of changes accompanying the resumption of growth should be sought first of all in the buds themselves, and not in the surrounding storage tissue.

5. It is pointed out that more work is needed in localizing these responses more closely *within the bud*.

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A STUDY OF THE METHOD OF SPORE GERMINATION IN MYXOMYCETES¹

FRANK A. GILBERT

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INTRODUCTION

Although the germination of myxomycetous spores was observed as early as the time of deBary (1, 2, 3), few investigators, even at present, agree upon the precise method by which the swarm cells emerge from the spore membranes.

According to Jahn (6) there are two possible methods: "Entweder könnte der eingeschlossene Protoplast die Membran an einer Stelle auflösen und durch das entstandene Loch hindurchschlüpfen, oder er könnte ohne irgend eine Verletzung der Membran, sozusagen auf rein physikalischen Wege einen so hohen osmotischen Druck entwickeln, dass die Hülle schliesslich zerreißt." Jahn states further that the cell wall ruptures by pressure from within but believes that an enzyme "glycogenase" plays a part in the process by changing glycogen in the spore to maltose and, since "der osmotisch wirksame Stoff im neubelebten Plasma ein Zucker ist," promotes germination.

Skupienski (12) frequently asserts that the germination of myxomycetous spores "n'est qu'un phénomène purement osmotique," agreeing thus far with Jahn, although entirely rejecting the latter's supposition concerning enzym action.

On the other hand, Constantineau (4) disagrees with both for he says, "Vom osmotischen Druck ist die Keimung unabhängig. Aus einem Vergleich der Keimung in isotonischen Lösungen ergibt sich ein Unterschied zu Gunsten des Zuckers."

Pinoy (9, 10, 11) considers that bacterial action is necessary to soften the wall and thus facilitate germination of the myxomycetous spores. This theory, however, is not generally accepted at present, as most investigators of the group have found no evidence to support it. Indeed, it has been clearly shown by the writer in the present paper, and by others (8, 12), that in certain species such as *Reticularia lycoperdon* and *Didymium nigripes*,

¹ Contribution no. 96 from the Cryptogamic Laboratories of Harvard University.

[The Journal for May (15: 277-344) was issued May 28, 1928.]

the spores may germinate in distilled water in less than an hour, a time so short that the very few bacteria present could have no appreciable influence in the process.

While studying spore germination in many species of Myxomycetes and watching the emergence of the swarm cells from the spore membranes, the writer has had opportunity to note certain differences in the manner in which the latter rupture or become torn and in the way that the swarm cells escape, and it is the purpose of the present paper to record these observations, to give a clearer conception of the more or less obscure process of germination, and to call attention to the fact that in certain species of the Physaraceae and Didymiaceae the spores give rise to from one to four swarm cells, rather than to the usual single swarmer.

METHODS OF GERMINATION

The fifty-six species and varieties of Myxomycetes used in these experiments were collected by the writer in eastern Massachusetts during the seasons of 1924, 1925, and 1926, and the spores were sown in distilled water in Syracuse glasses, the process of germination being observed by means of a Leitz star D water-immersion lens.

As a result of these observations, the writer finds that, while numerous species show transitional conditions, there appear to be two well-defined types of germination as stated by Jahn.² In the first, illustrated by *Fuligo septica*, the spore case ruptures by a deep wedge-shaped split which widens, allowing the content of the spore to push out, as the skin of an over-ripe grape might split on pressure, letting out the pulp; while in the second type of germination, as illustrated by *Dictydiaethalium plumbeum*, there appears in the wall a small jagged aperture, through which the swarm cell slowly creeps.

The germination of *Fuligo septica* (Pl. XXI, figs. 1-15) is as follows: The spores, which average 7.6 microns in diameter, germinate in from one to four hours. No apparent vacuole forms within the spore and the cell content remains practically undifferentiated from the time the spore is sown in water to the time the swarm cell emerges. The first indication of germination, therefore, is a slight cracking of the wall (fig. 3). The swelling of the protoplast within widens the gap and the split becomes gradually more pronounced (figs. 4-6). There is no slow flow of protoplasm through the aperture at this time, for the young swarm cell remains almost entirely within the wall of the spore. When the rupture has progressed sufficiently, the rounded swarm cell is carried away from the cell wall through no apparent movement of its own, for no flagellum is formed and there is no

² The two methods of germination were also noticed by deBary, for in "Die Mycetozoen," 1864, he says, after describing a germination similar to that of *Dictydiaethalium plumbeum*, "Das gleiche tritt an einer vorher nicht unterscheidbaren Stelle der Wand ein. Die Sporenmembrane von *Aethalium*, *Physarum albipes*, *Didymium praecox*, *Libertianum* u.s.w. reißt tief zweiklappig oder in mehreren Längsrissen auf."

noticeable movement of protoplasm (fig. 9). Occasionally the swarm cell is not moved away but remains quiescent, partly within the split spore wall (fig. 11), until sufficient time, usually ten or fifteen minutes, has elapsed for it to form a flagellum (fig. 10) and start the active movement characteristic of the swarm cell. The formation of the flagellum is in most cases preceded by a slow amoeboid movement, whether the swarm cell is free or partly within the cell wall. The degree to which the cell wall ruptures varies greatly (figs. 12-15) but sometimes the splitting continues until it is so complete as to divide the spore membrane into two or more parts.

In direct contrast to this first method of germination, as shown by the spores of *Fuligo septica*, is that of the spores of *Dictydiaethalium plumbeum* (Pl. XXI, figs. 16-30). After these spores, which average 9.45 microns in diameter, have been sown in water a varying length of time, usually from 3-12 hours, the uniform content becomes more noticeably granular and develops one or more small vacuoles, while sometimes slight spasmodic movements take place. A spore that is about to germinate may be distinguished as long as a half hour before the swarm cell actually emerges, by the formation of a large yellowish vacuole opposite the place in the wall where the content is to come out (figs. 18, 19). After this vacuole is formed, the first sign of actual germination is the emergence through the cell wall of a tiny papillate protuberance of protoplasm (fig. 20) which gradually grows larger (figs. 21, 22), the vacuole increasing in size at the same time and gradually occupying the space left as the content moves out (figs. 23, 24). When the mass has slowly emerged so that the greater part of it is outside, the remainder suddenly slides out, leaving the empty spore membrane or wall behind (fig. 25). The holes in the empty spore membranes of *Dictydiaethalium plumbeum* are not clean-cut and wedge-shaped but are roughly circular, with frayed or ragged edges (figs. 28-30) which, with methylene blue, stain much less deeply than the rest of the wall.

In the case of the first method, as exemplified by *Fuligo septica*, the wall ruptures by internal pressure rather than by enzym action. On the other hand it is difficult to understand how the second method of germination such as that of the spores of *Dictydiaethalium plumbeum* can be entirely the result of internal pressure. The small, ragged opening in the wall seems to indicate that the latter was softened and weakened in that region; while the behavior of the swarm cells in passing through the wall is strongly suggestive of that of the amoeboid swarmers of certain parasitic Chytridiales which pass from cell to cell of the host by dissolving a minute opening in the intervening wall.

The method of germination found to occur in most species of Myxomycetes falls into one or the other of these two categories, or in intergrading methods between the two clear-cut extremes. From the evidence gathered in studying the germination of a large number of representative species, it appears that spores of the sub-order Calcarineae,³ which includes the

³ The Physarales of MacBride's classification.

families Physaraceae and Didymiaceae, germinate chiefly in the manner of *Fuligo septica*, while the spores of the order Lamprosporales, for the most part, germinate like those of *Dictydiaethalium plumbeum*. It seems clear, therefore, that in the Myxomycetes as a whole, while spore germination is effected chiefly by internal pressure of the swelling protoplast, in certain species local enzym action on the wall may also play an important part.

SPORE GERMINATION OF *RETICULARIA LYCOPERDON* AND
LEOCARPUS FRAGILIS

Rather unusual methods of germination are shown by the spores of *Reticularia lycoperdon* and *Leocarpus fragilis*. The former germinate in a manner somewhat similar to that of the spores of *Dictydiaethalium plumbeum*, that is, by means of local softening and perforation of the wall (Pl. XXII, figs. 31-45), but in the former species the process is remarkable for the short time taken in its consummation. The spores usually germinate in less than a half hour at laboratory temperature and the writer has seen swarm cells emerge in from ten to fifteen minutes. A spore of *Reticularia lycoperdon* is reticulately sculptured over about two-thirds of its surface (fig. 31), the remainder being comparatively smooth and thin, and it is through the thin portion that the swarm cell escapes (figs. 37-39). The actual process of emergence takes but a few seconds; the thin part of the wall is stretched and rounded outward by internal pressure and finally ruptures (fig. 36), the swarm cell emerging rather suddenly and often with some force.

On the contrary, *Leocarpus fragilis* like *Fuligo septica* germinates by means of a wedge-shaped split in the wall (Pl. XXII, figs. 46-53) but the rupture is never so pronounced and the whole process is much slower, for the content may take two or three hours to escape. Chiefly, however, it is noteworthy that a spore gives rise to from one to four swarm cells, a characteristic that is apparently quite normal as it occurred in each of the eleven gatherings thus tested. It should be noted also that the spores were not multinucleate blocks, the result of imperfect cleavage, but were normal spores, perfectly formed, of equal or but slightly varying size.

As each spore so regularly gives rise to from one to four swarm cells, in all New England material at least, it seems strange that those who have germinated the species heretofore did not observe this condition. According to deBary (2), Hoffman obtained germination in this species, and Constantineau (4) in his work gives the percentage of germination as 55 percent, but as he does not describe the process, it seems probable that he overlooked the fact that more than one swarm cell may emerge from the spore, and means by his statement that, in fifty-five out of one hundred spores, he saw a splitting of the wall, or the empty spore cases, indicative of germination.

Moreover it was found that freshly formed spores of this species do not germinate so well as spores a few weeks old; although occasionally germi-

nation was obtained within a day or so after the sporangia had been gathered.

The percentage of germination was not found to be very high, forty to fifty percent on the average, assuming a germinated spore as one from which at least one swarm cell has emerged. With fresh spores no germination took place until two and one-half days from the time of being sown; but swarm cells from older spores were observed to emerge within twelve hours. The swarm cells come out very slowly, and under continuous observation the time required in one instance was two and one-half hours, and in another three hours, the movement being so gradual as to be hardly recognizable. Each of these swarm cells after freeing itself from the spore wall took about one and one-half hours to form a flagellum and move away.

At just what point division into individual swarm cells occurs is not known at present. Reduction division, in some species of the Endosporeae at least, and probably in all, has been found by various investigators to occur during the formation of the spores. If all of the spores of this species contained four swarm cells, it would seem probable that the spore wall formed about the mother cell rather than about each of the four daughter cells, as is usually the case; but since the spores contain from one to four swarmers, this does not seem probable. Whether or not there is a division of the protoplasm within the spore at some time, or whether the spore wall occasionally forms about more than one potential swarm cell, is not known; but there is no division of the protoplasm after the spores are fully matured, for as soon as such dried spores are sown in water the one to four swarm cells within may often be very clearly seen (figs. 48, 49). In a sowing of the spores of one specimen fifteen years old there was no germination, but the swarm cells could be seen as plainly as in the other younger specimens.

During the process of germination there is often residual protoplasm left in the cell. The two or more swarm cells that have emerged from the same spore membrane are not always of the same size; but each, of course, is smaller than a single swarm cell which corresponds to the whole content of a spore. However, small size does not appear to affect the vitality in any way and the lesser swarm cells soon grow to the size of their formerly larger companions. The swarm cells remain in the dancing stage but a short time, and are inclined to be somewhat longer and narrower than the ordinary swarmer; but as any swarm cell while creeping may elongate temporarily, no specific distinction may be attached to this peculiarity.

SPORE GERMINATION IN THE CALCARINEAE

Although *Leocarpus fragilis* has been considered in some detail, being perhaps the best specific example of a myxomycete the spores of which were found by the writer to give rise to more than one swarm cell, it is by no means the only one. In the sub-order Calcarineae of Lister's classification, which includes the families Physaraceae and Didymiaceae, he has observed

the germination of eighteen species, among which nine, including *Leocarpus fragilis*, show definitely that one normal spore may emit more than a single swarm cell (Pl. XXII); while in a number of other species, although emergence was not seen, there were strong indications that more than one swarmer in a single spore also occurred. These nine species are shown in table I.

TABLE I. *Number of Swarm Cells to a Spore in Certain Species of the Calcarineae*

Species	Number of Gatherings	Number of Swarm Cells to a Spore
<i>Badhamia lilacina</i>	2	I-4
" <i>magna</i>	I	I-4
<i>Physarum compressum</i>	I	I-2
" <i>connatum (notabile)</i>	2	I-4
" <i>leucopus</i>	I	I-2
" <i>serpula</i>	2	I-2
" <i>virescens</i>	I	I-2
<i>Leocarpus fragilis</i>	II	I-4
<i>Mucilago spongiosa</i>	I	I-4

Since this striking condition appears to be so general in the sub-order, it is remarkable that it has remained almost unnoted by previous investigators. DeBary (2), to be sure, reported that in *Didymium squamulosum* and *Didymium difforme* spores give rise to one or two swarm cells, but, save for this and a few other brief references (5, 7), this condition in the Myxomycetes seems to have been overlooked by most authors and it is still commonly stated that spores of the Endosporeae give rise to a single swarm cell. It is to be hoped that more extensive investigations will give additional information on this point in other members of the order from other parts of the world.

SUMMARY

In studying the germination of fifty-six representative species and varieties of Myxomycetes collected in New England in 1924-1926, the writer finds that there are two distinct methods of spore germination, one in which the swarm cell escapes through a deep wedge-shaped rupture in the spore wall, as in *Fuligo septica*; and one in which the swarm cell emerges through a small jagged aperture which appears in the wall, as in *Dictydiaethalium plumbeum*.

In the first case, spore germination, *i.e.*, emergence of the swarmer, is effected chiefly by rupture of the wall through internal pressure of the swelling protoplast, while in the second the softening of the wall substance by local enzym action seems to play an important part.

Spores of the Calcarineae appear to germinate like those of *Fuligo septica*; while, on the contrary, the manner of germination of the spores in the Lamprosporaes resembles more closely that of the spores of *Dictydiaethalium plumbeum*.

Spores of *Reticularia lycoperdon* germinate like those of *Dictydiaethalium plumbeum* but in the former species the process takes less than a half hour for its consummation.

Spores of *Leocarpus fragilis* give rise regularly to from one to four swarm cells, a condition apparently not reported heretofore in this common species.

Of the eighteen species of the Calcarineae in which germination has been observed, nine, including *Leocarpus fragilis*, show definitely that one normal spore may emit more than one swarm cell, usually from one to four; but this condition is apparently restricted to the two families which compose this sub-order.

The writer is greatly indebted for advice and criticism to Dr. William H. Weston, under whose supervision this work was carried out.

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EXPLANATION OF PLATES

All magnifications $\times 900$

PLATE XXI

FIGS. 1-15. Germination in *Fuligo septica*, illustrating the extreme type of germination in which the spore wall ruptures by internal pressure rather than by enzym action.

FIGS. 1, 2. Spores before germination.

FIG. 3. The first apparent stage in germination. The wall has started to split because of the increasing pressure of the protoplast.

FIGS. 4-8. Stages in germination illustrating the escape of the swarm cell or protoplast from the spore membrane.

FIG. 9. The swarm cell is free from the spore membrane but has not yet formed a flagellum.

FIG. 10. The swarm cell a few minutes later with the flagellum fully formed.

FIG. 11. Swarm cell which has remained temporarily within the spore membrane.

FIGS. 12-15. Empty spore membranes showing various ways in which they have split.

FIGS. 16-30. Germination in *Dictydiaethalium plumbeum*, illustrating the extreme type of germination in which the spore membrane is apparently softened locally by enzym action, permitting the protoplast to push out through the softened or weakened spot.

FIGS. 16, 17. Spores before germination.

FIG. 18. Spore showing the yellowish vacuole, the first noticeable indication of approaching germination.

FIG. 19. The vacuole has increased in size but the protoplast has not yet penetrated the wall.

FIG. 20. The vacuole has grown still larger and a small bit of protoplasm is protruding through the wall.

FIGS. 21-24. Later stages in germination showing the emergence of the protoplast or swarm cell.

FIG. 25. The swarm cell is free from the spore membrane but has not yet formed a flagellum.

FIGS. 26, 27. Young swarm cells with recently formed flagella, and spore cases from which they emerged.

FIGS. 28-30. Empty spore membranes showing characteristic frayed or ragged openings through which the swarm cells have emerged.

PLATE XXII

FIGS. 31-45. Germination of *Reticularia lycoperdon*.

FIGS. 31, 32. Spores before germination showing the partly reticulated surface and the smooth portion through which the swarm cell pushes out.

FIG. 33. Spore showing the slight swelling at the smooth area. This is the first indication of approaching germination.

FIGS. 34, 35. Later stages in germination showing the increased local swelling.

FIG. 36. The wall has ruptured at the smooth area and the swarm cell is about to escape.

FIGS. 37, 38. Escape of the protoplast or swarm cell.

FIGS. 39, 40. Young swarm cells just before the formation of the flagella, and spore membranes from which they have emerged.

FIG. 41. Young swarm cell after the formation of the flagellum.

FIG. 42. Empty spore membrane.

FIGS. 43, 44. Swarm cells.

FIG. 45. Encysted swarm cell.

FIGS. 46-53. Germination of *Leocarpus fragilis*.

FIG. 46. Ungerminated spore.

FIG. 47. Empty spore membrane.

FIG. 48. Spore before germination showing three enclosed swarm cells.

FIG. 49. Spore before germination showing two enclosed swarm cells.

FIG. 50. Spore giving rise to a single swarm cell, or protoplast.

FIG. 51. Spore membrane from which one swarm cell has emerged and which still contains a second.

FIG. 52. Spore membrane still containing three of the four original swarm cells; the fourth, having formed a flagellum, is active.

FIG. 53. Spore membrane still containing two of the three original swarm cells.

FIGS. 54-57. Germination in *Mucilago spongiosa*.

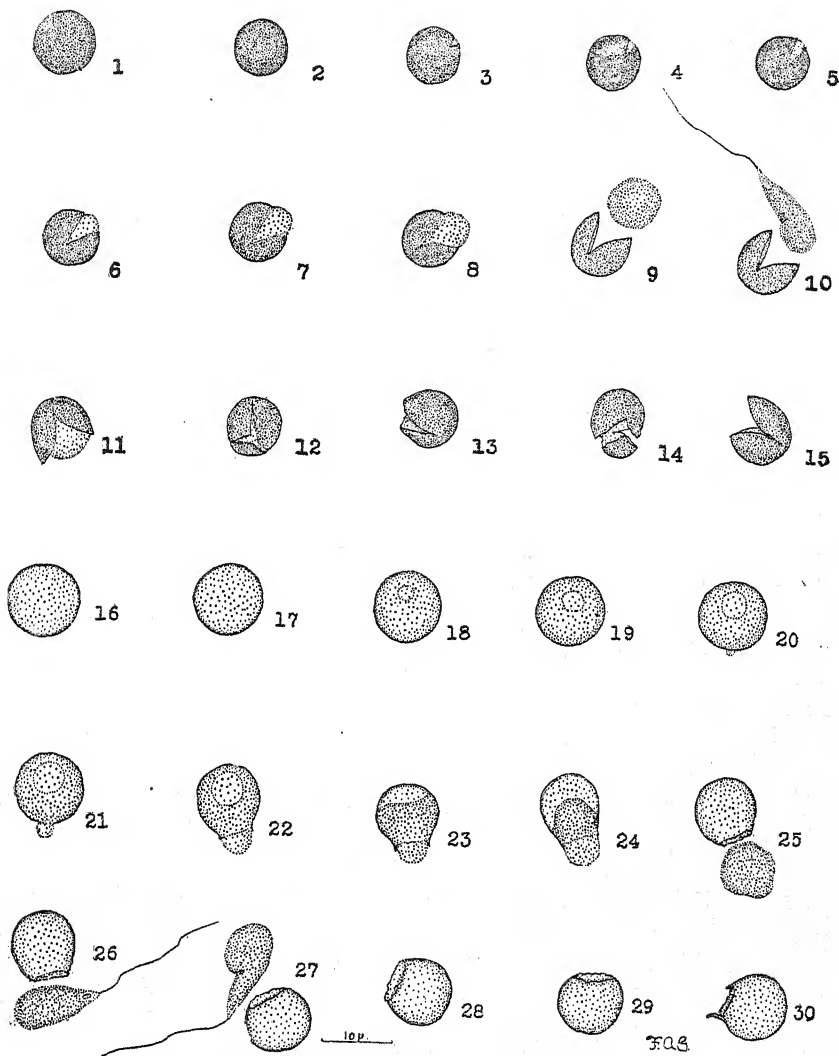
FIG. 54. Fractured spore membrane containing a bit of residual protoplasm. One of the original swarm cells is shown above and at the left of the spore membrane.

FIG. 55. Empty spore membrane.

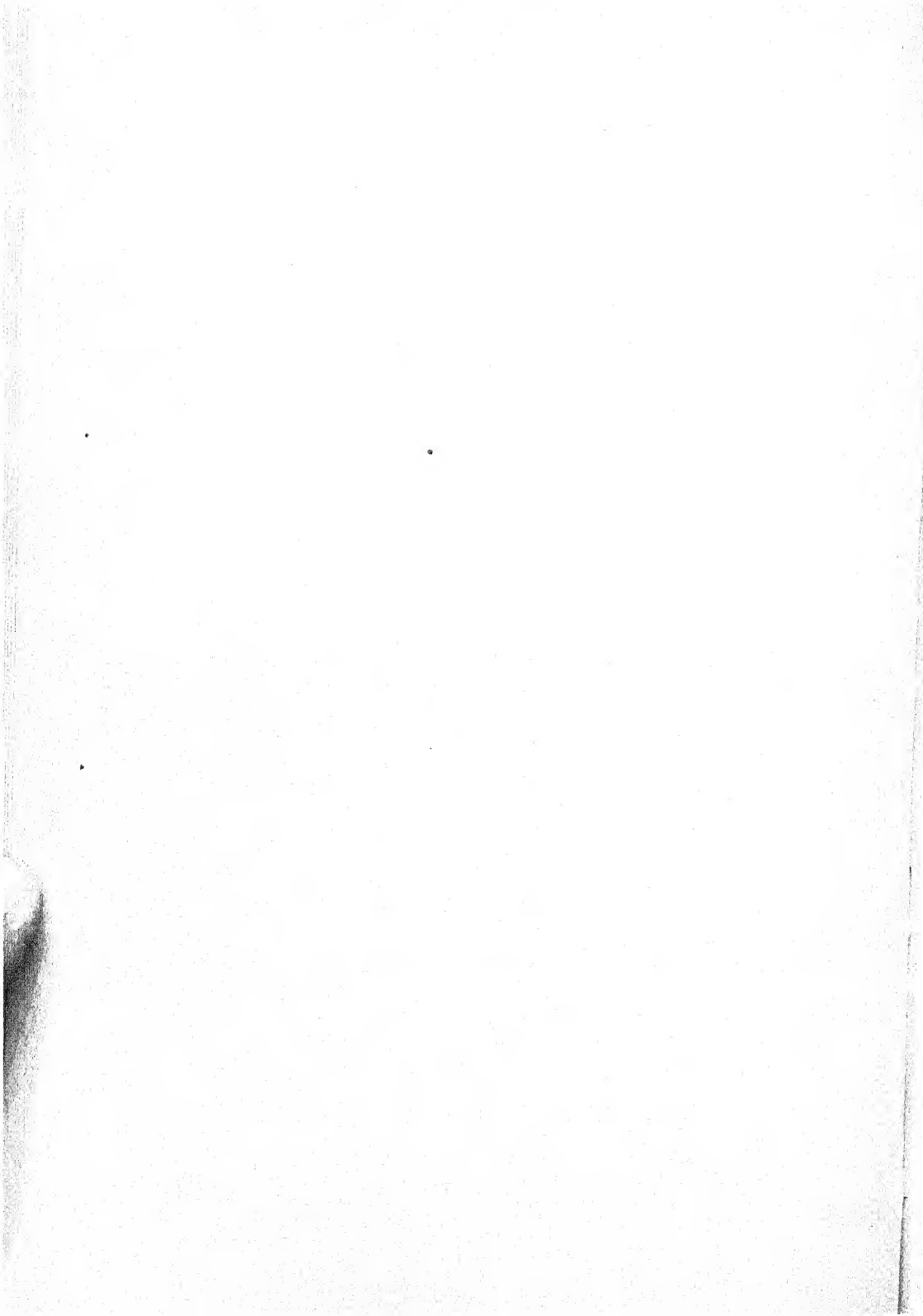
FIG. 56. Spore membrane containing two of the original swarm cells.

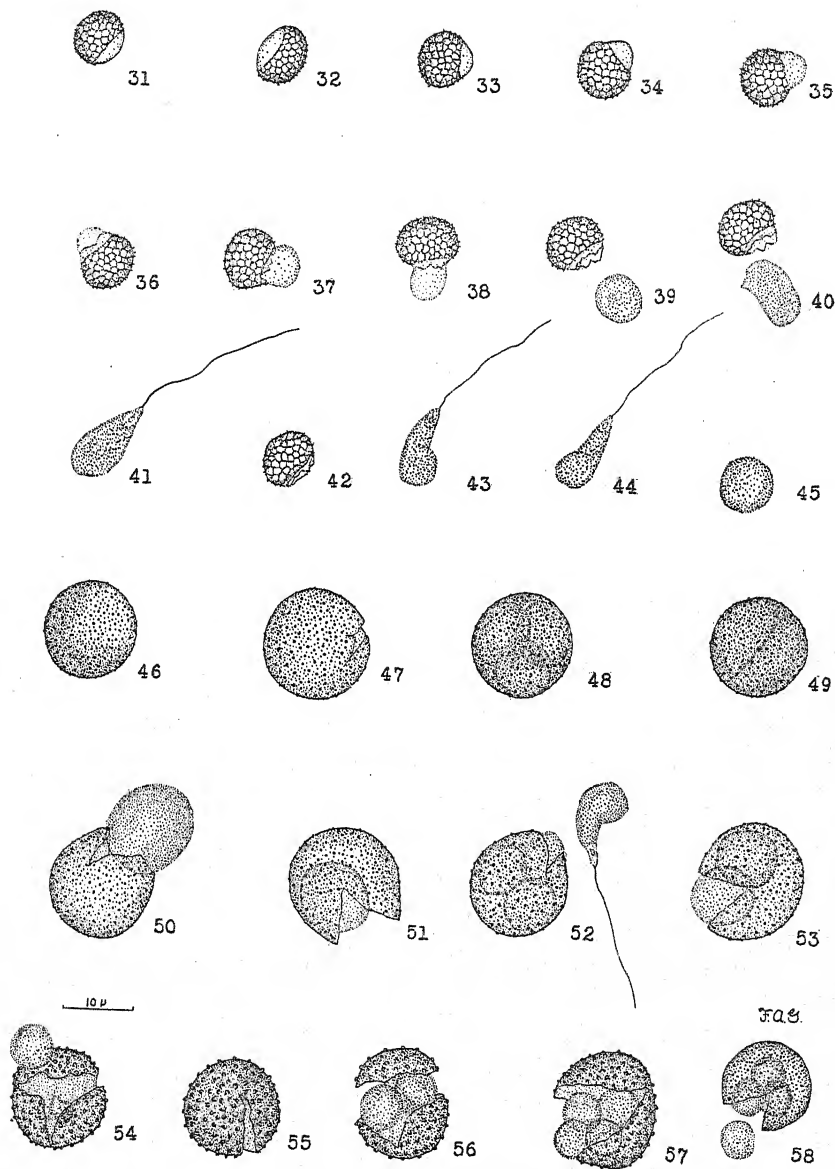
FIG. 57. Spore membrane containing four swarm cells.

FIG. 58. Germination in *Physarum connatum*, showing fractured spore membrane with four swarm cells.

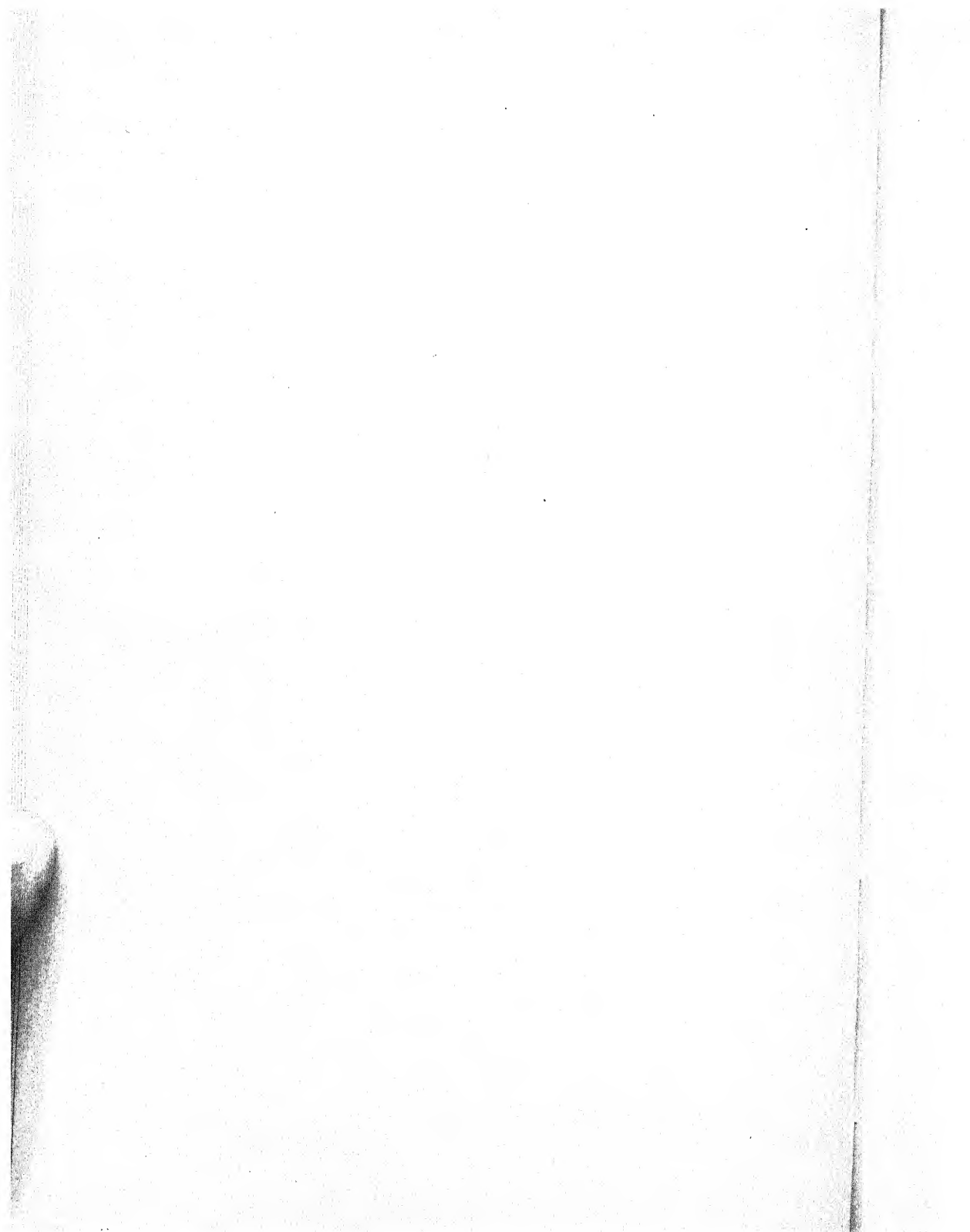


GILBERT: SPORE GERMINATION IN MYXOMYCETES





GILBERT: SPORE GERMINATION IN MYXOMYCETES



THE EFFECT OF THE SALT CONCENTRATION OF THE CULTURE SOLUTION ON THE GROWTH AND COM-POSITION OF PINEAPPLE PLANTS¹

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During studies involving the determination of the salt content of a number of field-grown pineapple plants, it was observed by the senior author that the salt concentration was not uniform. The fluctuations that developed were attributed, at that time, to the influence of the ages and growing localities of these plants. Two of the questions raised were: (1) Do the salt concentration and pH values of the soil solution influence those of the tissue fluids? (2) Does the salt concentration of a given soil solution, as determined by the electrical resistance method, indicate in any way its fertility or infertility, assuming that agricultural soils do not vary considerably in the kind of salts that enter the solution? As both these questions are of economic importance, the investigations presented in the succeeding pages were conducted.

METHODS

Crowns from pineapple fruits were selected to be as uniform as possible in weight, length of the fully developed leaves, diameter of the core, and other such characters. These were then stripped (freed from the basal vestigial leaves), cured (exposed to sunlight) for a week, and at the end of this period were planted, by being suspended one per jar, in stoneware jars containing nutrient solutions.

TABLE I. *Salt Content, Resistance in Ohms, and Average pH of the Different Series at 27-29° C.*

Series	Milligrams per Liter				Ohms	pH
	KH ₂ PO ₄	KNO ₃	MgSO ₄	Ca(NO ₃) ₂		
A.....	34,000	25,000	61,500	59,000	57.5	4.4
B.....	17,000	12,500	30,750	29,500	100.0	4.6
C.....	8,500	6,250	15,375	14,750	195.0	4.9
D.....	4,250	3,125	7,688	7,375	350.0	5.0
E.....	2,125	1,562	3,844	3,688	650.0	5.2
F.....	1,062	781	1,922	1,844	1,175.0	5.4
G.....	531	390	961	922	2,200.0	5.6
H.....	265	195	480	461	3,650.0	5.7
I.....	132	98	240	230	5,925.0	5.8
J.....	61	49	120	115	8,750.0	6.0
K.....	30	24	60	58	11,500.0	5.8
L.....	15	12	30	29	13,500.0	5.8

¹ Technical paper no. 3 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

There were twelve different nutrient solutions each with a different salt concentration. These solutions are designated as series *A, B, C, D, E, F, G, H, I, J, K, and L*. The chemical composition of each series or culture solution is given in table 1. The distilled water used measured 25,000 ohms and pH 6.5.

PLANT GROWTH AND BEHAVIOR

Ten plants were grown in each of the different culture solutions for nine months. The solutions were changed twice a month during the first five months and once a month during the last four. The electrical resistance and pH of the different series were determined three times on each individual solution, namely, when the new solution was placed in the jar, when the solution had reached the middle of its period, and when it was taken out of the jar. Few chemical analyses were conducted to find out what elements the plants absorbed from the solutions. The changes produced in the electrical resistance and pH of the different series have been considered as sufficient evidence of the effects of the plants on their solutions.

Table 2 gives an idea of the average behavior of the ten plants of each series in modifying the culture solutions.

TABLE 2. *Changes in the pH and Electrical Resistance at 15- and 30-Day Intervals*

Series	Ohms			pH		
	Initial	After 15 Days	After 30 Days	Initial	After 15 Days	After 30 Days
<i>A</i>	57.5	58.0	60.0	4.4	4.6	4.5
<i>B</i>	100.0	105.0	110.0	4.6	4.4	4.6
<i>C</i>	195.0	206.0	217.0	4.9	4.9	4.8
<i>D</i>	350.0	390.0	425.0	5.0	5.0	5.0
<i>E</i>	650.0	750.0	1,000.0	5.2	5.2	5.0
<i>F</i>	1,175.0	1,325.0	1,450.0	5.4	5.5	5.5
<i>G</i>	2,200.0	2,750.0	3,000.0	5.6	5.5	5.5
<i>H</i>	3,650.0	5,500.0	6,750.0	5.7	5.2	5.2
<i>I</i>	5,925.0	7,500.0	8,250.0	5.8	5.5	5.2
<i>J</i>	8,750.0	10,000.0	11,000.0	6.0	6.0	5.2
<i>K</i>	11,500.0	15,750.0	15,500.0	5.8	5.8	5.2
<i>L</i>	13,500.0	17,500.0	18,000.0	5.8	5.7	5.2

The plants of practically all the different series exerted some influence on the initial pH and electrical resistance of their solutions. In certain series the initial H-ion concentration was not changed, as in *A, B, C, and D*, but in others it was increased. The electrical resistance, however, increased throughout.

The growth of the plants varied considerably in the different series. Moreover, the resistance of the plants to disease appeared to be influenced by some of the culture solutions. For example, a number of the plants of series *C, D, E, F, G, and H* died after they had been growing for four or five months, but none of series *A, B, I, J, K, or L* did so. Susceptibility to disease in this particular case was correlated with rapid growth, while the

opposite was true for resistance. The plants of series *A* grew extremely slowly, showing no signs of growth during the first three months after planting. Those of series *B* were the next slowest growing. The plants of series *I*, *J*, *K*, and *L* made as rapid growth as those of *C*, *D*, *E*, *F*, *G*, and *H* during the first three or four months after planting, but thereafter grew extremely slowly or ceased altogether. In table 3 is recorded the average weight of ten plants in each series. In cases where some of the plants had died only those that survived were used for computing the average.

TABLE 3

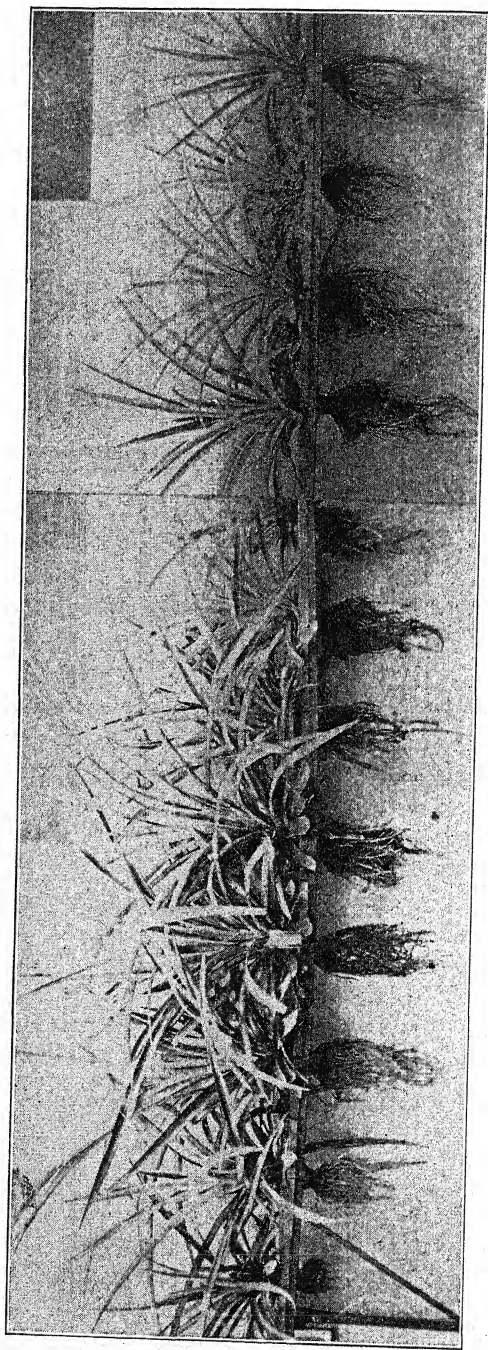
	Number of Plants Surviving	Average Weight in Grams *
<i>A</i>	10	500
<i>B</i>	10	900
<i>C</i>	8	1,250
<i>D</i>	7	1,500
<i>E</i>	7	1,800
<i>F</i>	6	1,200
<i>G</i>	6	900
<i>H</i>	7	700
<i>I</i>	10	900
<i>J</i>	10	600
<i>K</i>	10	450
<i>L</i>	8	400

* Green weight.

An idea of the growth of these plants may be obtained from text figure 1.

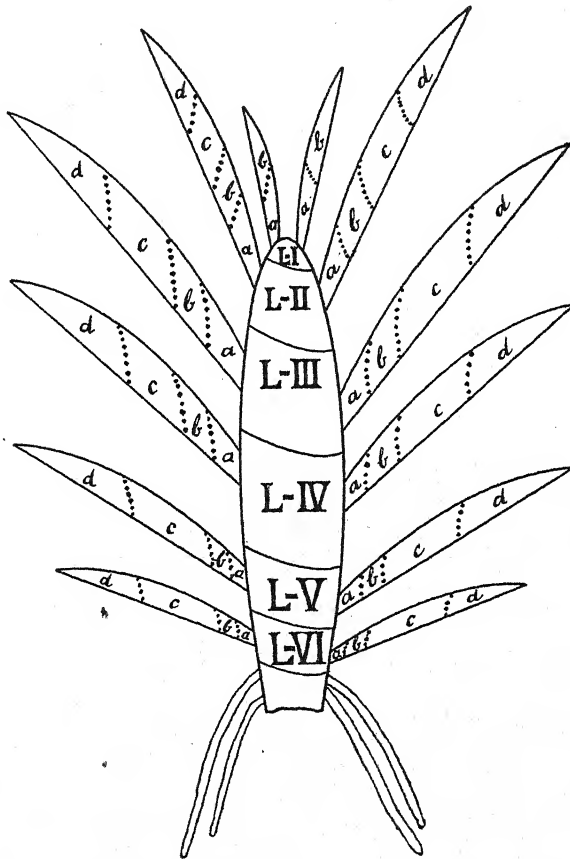
PHYSICOCHEMICAL PROPERTIES OF TISSUE FLUIDS

The following procedure was adopted for the study of the physicochemical properties of the tissue fluids of the plants. All of the plants of a series were taken from the jars and segregated into healthy and diseased individuals. Then the plants of each group were washed superficially with tap water to remove dust and other such adhering particles, then stripped, and the different parts grouped as outlined in text figure 2. After stripping, the members of each group were washed as thoroughly as possible in distilled water, the excess water being wiped off with a piece of cloth free from salt, acid, or alkali contaminations. The members of each group were then cut into as many pieces as indicated by the small letters of the alphabet in text figure 2. After cutting and subgrouping, the different subgroups were ground separately with a meat grinder. The pulpy mass that was obtained in each instance was pressed through cheesecloth and its fluid thus obtained. The expressed fluids were placed in the refrigerator for one or two hours to allow some of the particles to settle. The supernatant liquid from each was removed with a pipette and its electrical resistance and pH determined at about 28° C. \pm 1°. The electrical resistance of the tissue fluids was obtained on the Kohlrausch Wheatstone Bridge of a Leeds and Northrup Student Potentiometer connected to a 4-dial resistance box, and an A. C. galvanometer. A Henry type conductivity cell was used for the purpose.



TEXT FIG. 1. Representative plants of the series 4-L, left to right.

The H-ion concentration was determined electrometrically, using a quinhydrone electrode against a standard quinhydrone cell, instead of a calomel electrode connected to a Leeds and Northrup Student Potentiometer, according to Linderstrom-Lang's (23) method. The results of these determinations are recorded in tables 4 and 5. In table 6 are compared the results obtained from diseased and healthy plants.



TEXT FIG. 2. Diagram of a pineapple plant showing the various regions from which tissue fluids were extracted.

A few remarks on text figure 2 may be necessary to explain the manner in which the different parts of the pineapple plant were grouped and subgrouped. The leaves of the entire plant were divided into six main groups: the apical, or very young and usually semi-developed leaves, forming group L-I; the next group consisted of half to three-quarters developed leaves immediately adjacent to the above, forming group L-II, and so on. The number of leaves of each group varied considerably. The object of

grouping the leaves was to segregate leaves that were very old and had passed the prime of their functioning from leaves that were at their prime and fully functioning, and also very young leaves that had not yet reached their prime. The leaves of each such group were further subdivided according to the age of their tissues: subgroup *a* representing the youngest tissues, *b* tissues under differentiation, *c* fully developed and functioning tissues, and *d* the tip or oldest tissue of the leaf of fully developed plants. The latter subdivisions are shown in detail in text figure 2.

TABLE 4. *Electrical Resistance of Pineapple Tissue Fluids*

Plant Tissues	Ohms of the Plant Tissues of the Different Series											
	A	B	C	D	E	F	G	H	I	J	K	L
L-I, <i>a</i>	60.0	70.0	72.0	76.0	84.0	94.5	83.3	103.8	91.3	107.0	112.0	115.8
L-I, <i>b</i>	62.8	70.0	85.0	76.5	97.8	104.5	91.0	109.5	108.8	118.8	106.3	112.5
Average L-I...	61.4	70.0	78.5	76.3	90.9	99.5	87.2	106.7	100.0	112.9	109.2	114.2
L-II, <i>a</i>	50.0	54.3	59.3	70.0	82.0	91.3	80.0	105.0	88.0	101.3	99.5	103.8
L-II, <i>b</i>	54.3	64.3	64.0	75.0	89.0	100.8	88.8	109.5	95.8	116.3	105.5	112.0
L-II, <i>c</i>	56.3	54.0	61.5	73.3	86.8	98.8	86.3	105.5	101.3	114.3	97.3	115.0
L-II, <i>d</i>	55.5	55.5	63.0	65.9	77.8	88.8	86.5	88.0	94.5	93.0	85.8	101.3
Average L-II...	53.5	57.0	61.9	71.1	83.9	94.9	85.4	102.0	94.9	106.3	97.0	108.0
L-III, <i>a</i>	44.0	47.5	57.0	76.8	100.0	110.0	94.5	137.0	135.0	141.2	135.5	145.0
L-III, <i>b</i>	41.8	52.5	58.0	66.3	98.5	110.0	108.8	143.8	116.2	122.0	113.8	139.5
L-III, <i>c</i>	41.0	47.5	58.0	75.8	76.3	74.5	98.0	93.0	104.5	104.5	88.0	100.5
L-III, <i>d</i>	42.5	41.8	44.8	56.5	63.0	67.0	76.3	92.0	88.0	93.8	74.3	98.8
Average L-III...	42.8	45.7	51.8	64.4	84.3	90.8	88.5	117.5	108.0	115.4	102.9	120.9
L-IV, <i>a</i>	38.3	53.8	54.8	71.8	96.5	102.5	142.0	217.5	212.0	261.3	186.3	232.0
L-IV, <i>b</i>	37.5	46.8	49.5	57.5	81.8	88.8	108.8	166.3	161.3	189.5	141.0	170.0
L-IV, <i>c</i>	32.5	37.0	43.3	48.3	60.0	62.0	74.3	109.5	101.3	120.5	88.8	112.5
L-IV, <i>d</i>	32.5	37.0	42.3	46.3	58.3	53.8	61.3	87.5	98.8	111.3	81.2	106.3
Average L-IV...	35.2	43.6	47.5	56.0	74.1	76.8	96.6	145.2	143.2	170.6	124.3	155.2
L-V, <i>a</i>	34.8	58.3	58.0	71.8	98.8	132.0	164.5	277.5	215.0	320.0	165.0	228.8
L-V, <i>b</i>	33.8	44.8	52.3	61.5	84.0	98.8	113.8	234.5	180.8	263.0	157.5	195.0
L-V, <i>c</i>	34.8	38.5	48.0	52.5	65.7	76.3	80.5	144.5	156.2	183.8	118.8	146.8
L-V, <i>d</i>	34.8	36.5	49.5	56.3	67.3	81.2	82.0	125.5	127.0	153.8	123.2	151.3
Average L-V...	34.5	44.5	52.0	60.5	79.0	97.1	110.2	195.5	169.6	230.1	141.1	185.5
L-VI, <i>a</i>	34.8	46.8	74.8	78.8	95.3	136.8	130.0	214.5	180.8	dead	dead	dead
L-VI, <i>b</i>	33.5	44.3	55.8	71.7	78.5	100.0	113.0	195.0	162.5	"	"	"
L-VI, <i>c</i>	33.3	40.5	54.0	58.8	78.8	86.2	91.3	152.5	183.8	"	"	"
Average L-VI...	33.8	43.9	61.5	69.8	84.2	107.6	111.4	187.3	175.7	"	"	"
S-A.....	29.2	36.8	40.5	51.3	64.0	67.2	66.8	79.3	77.0	91.3	89.5	100.0
S-B.....	35.5	50.0	53.5	56.2	71.3	88.0	78.8	112.0	119.5	150.0	133.8	152.5
Average S.....	32.4	43.4	47.0	53.8	67.7	77.6	72.8	95.7	98.3	120.6	111.7	126.3
Roots.....	70.0	81.0	97.3	185.0	202.7	282.5	382.5	245.0	275.0	248.8	307.5	310.0

RESULTS

Salt Concentration

The results reported in table 4 indicate that the salt content of plant tissues is influenced considerably by that of the soil or culture solution.

In other words, the higher the salt concentration of a soil solution or a culture solution the more the plants grown in it will absorb. The condition may, therefore, be attributed to purely physicochemical forces. This point is emphasized considerably more when one considers the behavior of the plants of series A. These plants, as stated before, refused to show signs

TABLE 5. *Hydrogen-ion Concentration in pH Values of Pineapple Tissue Fluids*

Different Plant Tissues	Series of Solutions											
	A	B	C	D	E	F	G	H	I	J	K	L
L-I, <i>a</i>	5.70	6.20	6.00	5.90	5.85	6.70	5.75	5.35	5.60	5.90	5.60	5.65
L-I, <i>b</i>	5.50	6.10	5.05	6.45	5.90	5.80	5.90	4.90	5.55	5.80	5.85	5.85
Average of L-I.....	5.60	6.15	5.53	6.17	5.87	6.25	5.82	5.12	5.57	5.85	5.72	5.75
L-II, <i>a</i>	5.50	5.80	6.05	6.00	5.80	6.30	5.80	5.45	5.50	5.90	6.35	6.00
L-II, <i>b</i>	6.10	4.85	5.20	6.90	5.60	6.25	5.95	4.90	5.35	6.20	6.65	6.60
L-II, <i>c</i>	6.50	5.75	5.70	6.00	5.90	5.65	6.10	5.00	5.45	5.05	5.80	5.65
L-II, <i>d</i>		5.95	6.20	6.10	6.30	5.80	6.05	6.15	6.55	6.05	5.70	6.45
Average of L-II.....	6.30	5.59	5.79	6.25	5.90	6.00	5.97	5.37	5.71	5.92	6.12	6.17
L-III, <i>a</i>	5.45	5.55	5.65	5.60	5.50	5.00	4.90	5.05	5.05	4.90	5.40	5.45
L-III, <i>b</i>	6.50	5.55	5.40	5.10	6.10	5.00	5.45	5.10	5.35	5.75	6.10	6.05
L-III, <i>c</i>	6.50	6.40	6.20	6.55	6.65	6.00	6.00	5.70	6.10	4.60	6.35	6.55
L-III, <i>d</i>		6.80	6.70	6.90	7.35	6.30	5.90	6.05	6.55	5.90	6.30	6.45
Average of L-III.....	6.15	6.07	5.99	6.03	6.40	5.58	5.56	5.47	5.76	5.29	6.04	6.15
L-IV, <i>a</i>	5.40	4.60	5.05	5.30	5.20	4.70	4.80	4.60	4.45	4.50	5.15	5.00
L-IV, <i>b</i>	6.30	6.05	5.75	5.80	5.90	5.10	5.10	4.75	6.30	6.35	5.70	5.80
L-IV, <i>c</i>	6.80	6.35	6.65	6.90	7.10	6.35	6.20	5.90	6.45	6.30	6.45	6.80
L-IV, <i>d</i>	7.30	7.00	6.75	7.05	7.10	6.65	7.10	6.35	6.55	6.20	6.55	7.00
Average of L-IV.....	6.45	6.00	6.05	6.26	6.32	5.70	5.80	5.40	5.94	5.84	5.96	6.15
L-V, <i>a</i>	5.20	5.00	4.85	5.00	4.95	5.00	4.85	4.60	4.50	4.55	5.15	4.70
L-V, <i>b</i>	4.60	4.90	5.05	5.10	5.10	5.35	6.30	4.65	5.70	4.50	5.90	6.50
L-V, <i>c</i>	6.45	6.65	6.50	7.20	6.70	6.10	6.75	5.80	6.60	6.40	6.45	6.70
L-V, <i>d</i>	6.85	6.90	6.15	7.15	7.40	5.90	6.90	5.60	6.30	6.20	6.80	6.85
Average of L-V.....	5.78	5.86	5.64	6.11	6.04	5.59	6.20	5.16	5.78	5.41	6.07	6.19
L-VI, <i>a</i>	5.05	4.60	4.90	5.00	4.55	4.70	4.70	4.05	4.45	dead	dead	dead
L-VI, <i>b</i>	4.45	4.85	4.90	5.25	4.80	5.10	4.50	4.45	4.35	"	"	"
L-VI, <i>c</i>	6.45	5.95	5.90	6.90	7.15	6.30	6.55	6.00	6.75	"	"	"
Average of L-VI.....	5.31	5.13	5.20	5.71	5.50	5.34	5.25	4.83	5.18	"	"	"
S-A.....	5.45	5.30	5.40	5.65	5.85	6.30	5.60	5.30	4.95	5.20	4.95	5.50
S-B.....	5.40	5.25	5.20	5.60	5.60	5.80	5.20	4.80	5.00	4.95	4.80	4.85
Average S.....	5.42	5.27	5.30	5.63	5.72	6.05	5.40	5.05	4.97	5.07	4.87	5.17
Roots.....	4.85	5.30	5.15	5.80	6.15	6.20	5.70	5.30	4.50	5.15	5.10	5.05

of growth for almost three months in spite of the fact that their basal ends were suspended in an aqueous solution during all this period. This may be explained as follows: The salt concentration of the culture solution being almost the same as that of the tissue fluids had no influence whatsoever on the development of osmotic forces. As a result of this condition, the plants were not able to absorb water or salts. With the passing of the time, however, both the leaves and upper portion of the stem lost some moisture,

which condition increased the osmotic concentration of the tissue fluids. With the absorption of moisture the normal physiological processes of the plant begin. It seems that a continuous stream of water has to go from the outside solution into the plant body to keep the latter functioning properly. Plants are able to maintain the flow of this stream by means of

TABLE 6. *Electrical Resistance in Ohms of the Tissue Fluids of Healthy and Diseased Plants*

Plant Tissues	Series C		Series D	
	Healthy	Diseased	Healthy	Diseased
L-I, <i>a</i>	72.0	46.3	76.0	51.3
L-I, <i>b</i>	85.0	48.0	76.5	56.3
Average L-I.....	78.5	47.2	76.3	53.8
L-II, <i>a</i>	59.3	43.5	70.0	58.0
L-II, <i>b</i>	64.0	44.8	75.0	54.3
L-II, <i>c</i>	61.5	42.0	73.3	53.8
L-II, <i>d</i>	63.0	46.3	65.9	60.3
Average L-II.....	61.9	44.2	71.1	56.6
L-III, <i>a</i>	57.0	42.8	76.8	56.3
L-III, <i>b</i>	58.0	38.5	66.3	46.0
L-III, <i>c</i>	47.5	39.0	58.0	45.5
L-III, <i>d</i>	44.8	38.5	56.5	43.8
Average L-III.....	51.8	39.7	64.4	47.9
L-IV, <i>a</i>	54.8	37.3	71.8	42.8
L-IV, <i>b</i>	49.5	32.3	57.5	41.5
L-IV, <i>c</i>	43.3	34.5	48.3	37.3
L-IV, <i>d</i>	42.3	33.3	46.3	40.5
Average L-IV.....	47.5	34.3	56.0	40.5
L-V, <i>a</i>	58.0	39.3	71.8	44.5
L-V, <i>b</i>	52.3	31.3	61.5	41.3
L-V, <i>c</i>	48.0	33.3	52.5	37.5
L-V, <i>d</i>	49.5	36.8	56.3	40.0
Average L-V.....	52.0	35.2	60.5	40.8
L-VI, <i>a</i>	74.8	40.3	78.8	54.5
L-VI, <i>b</i>	55.8	34.3	71.7	53.5
L-VI, <i>c</i>	54.0	35.8	58.8	46.5
Average L-VI.....	61.5	36.6	69.8	51.5
S-A.....	40.5	33.8	51.3	41.3
S-B.....	53.5	35.5	56.2	47.3
Average S.....	47.0	34.7	53.8	44.3
Roots.....	97.3		185.0	

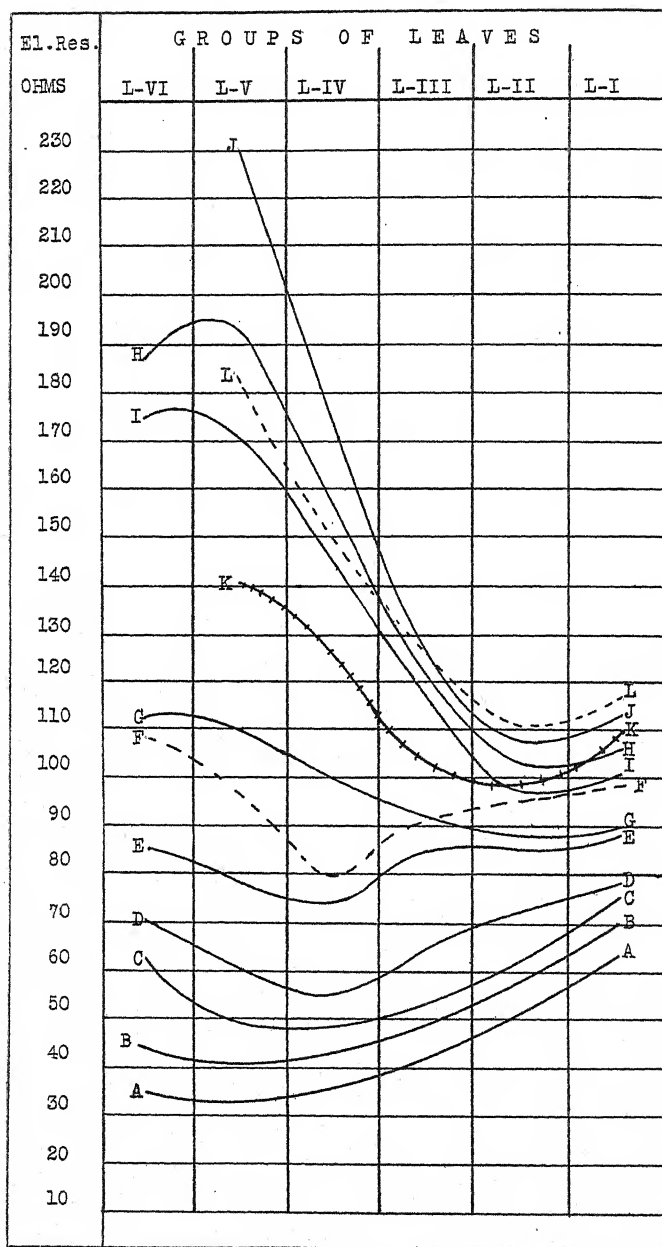
the high salt content of their tissues. There is only one exception out of almost a thousand cases where the salt content of the soil solution was found to be greater than that of the pineapple tissue fluids. This soil, from Waianae, showed an electrical resistance of 50 ohms, that of the tissue fluids averaged about 60 ohms. The plants in this particular soil were not able to grow at all when first planted, but after the soil had been exposed to rain and other climatic conditions for a few months and some

of the salts had leached out the land was replanted. The replants or new plants made a fair growth. The electrical resistance of the majority of pineapple soils varies between 300 and 5,000 ohms. There are many soils that have values ranging between 5,000 and 15,000 ohms, but these may be considered as very depleted and unfit for profitable agricultural use unless they receive heavy applications of fertilizers.

The plants of series *B* responded more readily than those of series *A*; root germination and growth progressed sufficiently during the first three or four weeks after planting. This was also true of the plants of all the other series. These plants made a rapid growth because they were able to absorb water readily on account of the higher salt concentration of their tissue fluids as compared with that of the water-culture solution.

It has been mentioned before that some of the plants of series *C*, *D*, *E*, *F*, *G*, and *H* died after a few months of rapid growth, but none died in series *A*, *B*, *I*, *J*, *K*, or *L* during their early growth. Some plants of series *L* died during the last part of the experiment, that is, after the plants had reached an age of eight months. The death of the plants in every case was due to root rot caused by species of *Fusarium*, judging from the mycological and bacteriological examination of the diseased tissues. As far as observations have gone one may be justified in attributing the high susceptibility to disease of the plants of series *C*, *D*, *E*, *F*, *G*, and *H* to the rapid growth of their root systems. That too rapidly growing tissues lack in disease resistance, owing to a number of histological and physiological causes, is well established.

With plants of series *I*, *J*, *K*, and *L* the results were different from any of the other series. These plants developed almost as rapidly as those of series *C* to *H* during the first three to four months after planting. At the end of this period, however, they suddenly slowed down in their growth, increasing only slightly or not at all between the fifth and ninth months of their growing period. There is no apparent reason why these plants were able to make such a great growth during the early period and little or none later since the salt concentration of the culture solution was maintained at the same point throughout the entire period of the experiment. The only plausible explanation is that suggested by the curves on text figure 3. The graph shows that the greatest accumulation of salts occurs in the leaves most active physiologically, such as those of groups L-II and L-III. Very old leaves, that is, those that have passed the prime of their activity and become senile, such as of groups L-IV, L-V, and L-VI, also lose some of their salt content. On the other hand very young leaves, such as of group L-I, possess a lower salt concentration than somewhat older leaves. The curves indicate that there is a possibility of translocation of salts from very old and less active leaves to younger and more active ones. Text figure 3 shows that the older leaves of the plants of all the series except *A* and *B* surrendered some of their salt content to the immediately adjacent younger



TEXT FIG. 3. Graph showing the electrical resistance of tissue fluids of the leaves of pineapple plants grown in the series of solutions described in table 1.

leaves. This is shown by the drop in the electrical resistance of the tissue fluids. The transfer of salts from senile to very active leaves increases as the salt content of the outside culture solution decreases. With cultures *H*, *I*, *J*, *K*, and *L* the curve becomes very steep, the older leaves of the plants involved surrendering about two-thirds of their initial salt content to the younger active leaves. The death of the leaves of group L-VI of the plants of series *J*, *K*, and *L* may be due to salt starvation, the leaves dying after having surrendered most of their salt content to the younger leaves and becoming thereby physiologically inactive. The arrested growth of the plants of *I*, *J*, *K*, and *L* after the fourth or fifth month from their planting may be attributed, with a considerable degree of safety, to an insufficient salt concentration in the culture solution. The plants made a very good growth during the first four to five months of their planting because the stored salts in the tissues of the planting material together with the salts of the culture solution were sufficient for the growth of the plants up to a certain stage, but not after these plants had increased considerably in size and the salts had become reduced in the tissues. One may raise the following question: Has this condition developed as the result of insufficient inorganic elements, of organic nutrients, or of accessory elements such as silica, manganese, boron, etc.? The results indicate that it is due to an insufficient supply of inorganic salts, because wherever such salts have been added in a sufficient concentration stunting did not develop. Apparently the concentrations at which the different elements were supplied in cultures *I*, *J*, *K*, and *L* was extremely low and possibly unavailable to the plants, if one considers that the water of the culture solution must have exerted some osmotic force on these salts. In series *K* and *L* there is a possibility that some exosmosis may also have developed, probably not from healthy tissues but from dead root tissues, accepting the evidence of Stiles (23). The conclusion to be drawn from these observations is that the arrested growth of the plants of series *I*, *J*, *K*, and *L* was due to an insufficient concentration of salts in their culture solutions. The behavior of these plants may be compared to field conditions sometimes encountered. The writer has occasionally observed plants in very poor soils where their early growth is good but becomes arrested and finally ceases after they have reached a certain stage.

The results obtained on the electrical resistance of the tissue fluids emphasize the point that pineapple plants require a more or less constant salt concentration in the leaves of greatest photosynthetic activity for normal functioning. In case such a salt concentration in these active leaves is difficult to obtain, due to the poor salt concentration of the culture solution, the older leaves surrender their salt content to the more active ones to make up the deficiency. In cases, however, where the salt concentration of the culture solution is higher than that required by the leaves of group L-III, the salt concentration of the leaves may be increased to a certain extent but not to as much as that of the very old leaves.

The behavior of the very old leaves toward those of prime physiological activity, *e.g.* group L-III, in plants grown in cultures of very low or very high salt concentrations may be utilized for determining cases of under-nutrition or over-nutrition of plants. The ratio of the electrical resistance of the fluids of very old and young leaves of the same plant should give the salt absorption coefficient. If we let y represent the electrical resistance of the fluids of young leaves and s that of old leaves, we have the following conditions:

- (1) $y/s = 1$ (normal salt absorption).
- (2) $y/s = 1$ plus (over-nutrition).
- (3) $y/s = 1$ minus (under-nutrition).

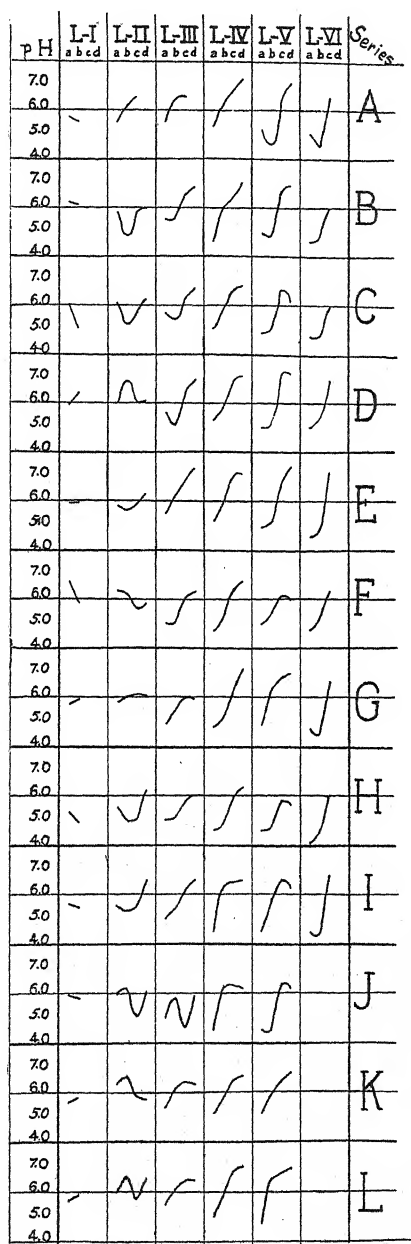
To what an extent the above findings are applicable to plants growing in soils, it is difficult to say, but the writers are working on this phase of the problem. The indications are that determinations of over-nutrition or under-nutrition are possible, but there are a number of difficulties associated with such determinations, particularly where diseases due to parasites affect the plants.

Hydrogen-ion Concentration

There was no influence whatsoever on the pH of the tissue fluids of the plants by the pH of the external solution, as shown in table 5 and text figure 4. The pH value of the fluids of different tissues of the same organ of the plant varies considerably. The differences in the tissues of very young leaves, such as of groups L-I and L-II, are not well defined, but those of the older leaves are very pronounced. The pH values of the tissue fluids of the leaves of groups L-III, L-IV, L-V, and L-VI, as shown in text figure 4, follow a definite curve. The tissue of greatest acidity is that on the border line between the white and green tissue of the leaf; the pH rising on either side of this tissue, that is, toward the base and apex of the leaf. This tissue indicated in table 5 and text figure 2 by b changes in acidity in the different leaves of the same plant, being more acid in the older than in the younger leaves. Judging from its behavior it may be of great importance to the physiological processes of the plant.

Healthy and Diseased Plants

Table 6 gives a very good picture of the conditions that may develop in a plant whose root system has ceased to function. The plants in this case not only did not absorb water after their roots had died, but lost considerable quantities of that which had entered their tissues previous to the death of the roots. In table 6 the loss of moisture from the tissues of healthy and diseased plants is shown clearly by the differences in the electrical resistance of the fluids when these plants are grown in the same culture solution. The loss in moisture of the diseased plants was also obvious from the wilted appearance of the leaves and other similar features.



TEXT FIG. 4. Graph showing the pH of the leaf tissue fluid of pineapple plants of series A to L.

This indicates that the root system is the only water-absorbing agent of the plant. The small amount of water that may enter the tissues suspended in water is not enough to maintain the initial turgor. This leads us to conclude that if pineapple plants have lost their roots they are bound to develop the flaccid or wilted condition regardless of how wet the soil in which they are grown may be. The tissues of the stem do not absorb very much water, or at least not enough to compensate for the amount lost through transpiration.

DISCUSSION

The results obtained on the physicochemical properties of the tissue fluids of the plants of the different series prove, on the one hand, that the salt concentration of some of the tissue fluids is not definite, but that it may vary between 5 and 500 percent or more, depending on the salt concentration of the soil solution which bathes the roots of the plants. On the other, however, they show how the salts are distributed in the plant body; that is, whether they accumulate in greater concentration in certain organs or in certain of the tissues of these organs.

Some of the tissues of the same organ are more acid than others, and those of a lesser acidity contain also a higher concentration of salts (text fig. 4 and table 5). Moreover, the tissues that show greater acidity are the young or recently developed or developing tissues, and generally tissues that are undergoing differentiation; the older tissues possessing, in the majority of cases, a higher pH value (text fig. 4). The only theoretical explanation for this that the writer has to suggest is that during the synthesis of new tissues certain acids are released by the protoplasm and that these serve as the main source for the generation of hydrogen ions. As tissues, however, become older and older with time and are in contact with the salts that have entered the tissues from the outside, they interact and some of the acids mentioned become neutralized. This becomes more clear when one takes into account the fate of the anions, such as NO_3^- , SO_4^{--} , and PO_4^{--} , that enter the solution with some of their corresponding cations, such as K^+ , Ca^{++} , Mg^{++} , and Fe^{++} or Fe^{+++} . The anions being synthesized into proteins leave the cations free in the solution to react with some of these acids, such as pectic, oxalic, citric, carbonic, etc., to form their corresponding salts. Such conditions are known to develop in plant tissues and there is no doubt that they are the source of such hydrogen-ion potentials as are recorded in text figure 4 and table 5. That the protoplasm may release acids or other substances that may be considered as by-products of protoplasmic synthesis is possible. Stewart (22) found in the case of cacti that mucilage is secreted by the protoplasm. Commenting on the development of hydrogen-ion potentials in biology Clark and his associates (3) make the following statement:

From the fact that acidic and basic groups are created or destroyed in the reactions of oxidation-reduction, it follows that the oxidation or reduction of a system plays a part

in the acid-base equilibria of a solution. We have been discussing this from the point of view of controlled pH values. We may now reverse the point of view and emphasize the fact that oxidation or reduction will displace pH in one direction or another in accordance with the acidic or basic nature of the group created or destroyed. Oxidation-reduction systems and acid-base systems are intimately related.

From this and other papers of Clark and other workers, it becomes evident that the changes in the acidity of tissue fluids are due, at least in part, to oxidation-reduction reactions.

The more acidic condition of young tissues has been observed by a number of other investigators. Astruc (1) has published a very extensive account of the acidic properties of plant tissues based on more than thirty different species of plants belonging to different families. From the information gathered he arrives at the conclusion that the younger tissues are more acid than the older. Gustafson (4) in his studies on the acidity of the different tissues of corn, squash, and sunflower concluded that there is a gradient of hydrogen ions from the base up to the apex of the stem. Small (20), Rea and Small (16, 17, 18), and Martin (9, 10) have found that different tissues of the same organ of a plant possess widely varying degrees of acidity. They also found that the acidity of such tissues is subject to variation depending on age of plant and season of year. Pearsall and Priestley (13) and Pearsall (14) have found that the pH of newly formed cork cells is very low, about 3.0, and that within the phellogen the pH of the cortex parenchyma is 5.5-6.5. They also found that the xylem has a pH varying between 4.3 and 5.0, and the phloem between 7.0 and 8.0. These writers make interesting deductions from such findings. They believe that due to the hydrogen-ion potential that exists between the phloem and xylem, which crosses the cambial layer, the synthesis of tissues becomes possible. They base their assertion on the following theoretical grounds:

One of the essential conditions for the development of dividing cells in normal non-growing tissues is the development of a gradient of hydrogen-ion concentration such that the protoplasm or its principal proteins can be reduced to an iso-electric point; this being the point at which proteins show minimum swelling, minimum osmotic pressure, maximum precipitation, and least viscosity. It may therefore be assumed that if the protoplasm or the principal proteins in a tissue were made approximately iso-electric by an alteration in H-ion concentration, then they would tend to lose water to adjacent media of higher osmotic potential, and as a result the condition in the protoplasmic protein aggregate would favor synthesis. Since proteins are least soluble at the iso-electric point, the formation of additional protein would be favored at this point by the removal of the end products of the reaction.

Our explanation of the changes in the acidity of the tissue fluids as due to the neutralizing effect of cations, which reaction would involve a transfer of electrons in the involved ions and the establishment of an oxidation-reduction system, is therefore in agreement with Clark's theory. We are not in a position to say that the formation of acids in very young tissues

has anything to do with synthesis. Pearsall and Priestley's theoretical explanation is, however, logical and convincing.

The literature on the effect of environmental conditions on the chemical composition of plant tissues is not very extensive. Some workers in both Europe and America have conducted investigations on plants of the same species grown in different localities. Others, however, grew plants under controlled conditions and then studied the composition of the plant tissues. Hoagland and Davis (7) in their studies on the relation of absorbed ions to the composition of the cell sap found that penetration of ions into cells may take place from a solution of low concentration to one of a higher concentration and that nearly all of the inorganic elements that are present in the cell sap exist in ionic state. Osterhout (12), discussing his findings on the importance of maintaining certain differences between cell sap and external medium, arrives at the conclusion that the sap of *Valonia macrophysa* is not a balanced solution in the ordinary sense, and the question may be raised whether, in general, the interior of the cell requires a balanced solution in order to maintain life. It may be that we must distinguish between internal and external balanced solutions. The writers have given a slightly different interpretation of their findings in Series A than did Osterhout. Mason (11) who studied the factors affecting the concentration of electrolytes in the leaf sap of *Syringa* arrives at the conclusion that there is a tendency for the concentration of electrolytes to vary inversely with that of non-electrolytes and that such fluctuations are associated with the rate of carbon assimilation, which determines, in turn, the rate at which such electrolytes are removed from solution in metabolism. The same author has also found that the electrolyte content of the cell sap may vary with the different locations where the plants are growing. Sprecher (21), who studied the osmotic pressure of both green and mosaic plants of two species of *Tropaeolum* (*T. lobbianum* and *T. majus nanum*), found that the osmotic pressure of the cell sap was influenced by humidity, air, heat, illumination, and time. For example, he found that osmotic pressure was least in the morning hours, highest in the afternoon, and then lowered gradually to the forenoon medium. Absence of light produced a diminution of osmotic pressure corresponding naturally to the lowering of assimilation. Harris, Gortner, and Lawrence (5) found that the electrical resistance of the tissue fluids of ligneous plants is higher than that of herbaceous plants. Other work (6) by the latter authors presents evidence that the salt concentration of plants of the same species grown in the same soil may vary during the different seasons of the year. Reed and Halma (19) found in their studies on the relations between growth and sap concentration in citrus trees that the concentration of the sap obtained from fruit wood was generally higher than that from shoot wood of similar age. Also determinations of the sap concentration of thrifty and of unthrifty orange trees showed a higher sap concentration for the thrifty trees. The hydrogen-

ion concentration was found to increase during the period of rapid vegetative growth, when presumably carbohydrates are being extensively oxidized.

The above authors have observed that low osmotic concentrations of plant sap in tissues are correlated with high rates of growth. This is also true in the case of pineapples, as table 4 indicates, the salt concentration of the tissue fluids of the youngest leaves, group L-I, being lower than that of the somewhat older or fully developed leaves.

SUMMARY

1. The plants that were grown in the cultures of higher salt concentration absorbed more salts than those that were in cultures of low salt concentration. A very high salt concentration in the culture solution will increase that of the tissue fluids of young but fully developed leaves between 25 and 50 percent, and that of the very old leaves between 50 and 150 percent. A very low salt concentration, on the other hand, will decrease that of the tissue fluids of young but fully developed leaves between 25 and 50 percent, and that of the very old leaves between 50 and 750 percent. What happens apparently is that in cases of high salt concentrations in the outside solution the oldest leaves are used as storage organs for the excess of salt, that is, the amount over that necessary for the normal functioning of the young but fully developed leaves. In cases of low salt concentration in the outside solution, the stored-up salts of the oldest leaves are surrendered to the young but fully developed leaves to make up for their salt deficiency.

2. The pH of the external solution had no influence on that of the tissue fluids.

3. The plants that were grown in culture solutions of equal salt concentration to that of the tissue fluids, or higher, grew very slowly and then only after they had been in the solution for almost two months.

4. The plants that were growing in culture solutions the salt concentrations of which were considerably lower than those of the tissue fluids made a very rapid growth, which was followed by a very marked retardation in those cultures of very low salt concentration.

5. The plants that were grown in culture solutions that contained neither a very high nor a very low concentration of salts developed best. Some of these plants, however, were susceptible to fungal invasion, due apparently to the softness of the tissues resulting from a very rapid growth.

6. The salt content of the older tissues of the same organ is higher than that of the younger tissues; this the authors believe to be due to the longer contact of such tissues with the salts of the cell sap.

7. The salt content of the leaves at different stages of their development varies considerably. The youngest leaves, that is, those that are in the period of active growth and development, contain a lower salt concentration than the somewhat older or oldest leaves. The salt content of the oldest leaves is extremely variable. With senile leaves of plants growing in

culture solutions of high salt concentration the salt content is very high, and it is very low with those in cultures of very low salt concentrations. The only leaves that tend to maintain a more or less uniform salt concentration are those that have attained full development and are at the prime of their functioning. The salt content of this group of leaves may vary from 50 to 100 percent in extreme conditions, whereas under normal conditions this variation rarely exceeds 20 percent. Variations, on the other hand, in the salt content of very old leaves may be as great as 700 percent.

8. The acidity of the tissue fluids from different parts of the same organ of a given plant varied considerably. The portion of the tissue lying on the border line between the white and green tissue of the leaf was found to possess the highest degree of acidity, both the white toward the base and the green toward the tip being considerably less acidic in reaction.

9. The acidity of the tissue fluids of different leaves varied considerably.

10. By making use of the salt concentration of the oldest leaves compared with those of the young but fully developed leaves, when the plants are grown in cultures of either high or low salt concentration, it is believed that a coefficient of salt absorption may be found which is correlated with the over-nutrition, under-nutrition, or normal nutrition of pineapple plants.

The writers are indebted to Dr. A. L. Dean for reading the manuscript and for helpful suggestions.

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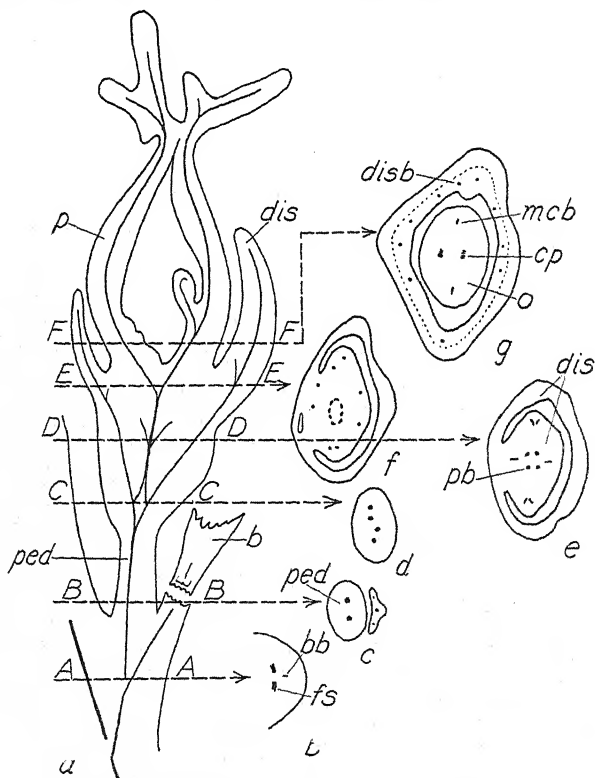
THE MORPHOLOGY AND ANATOMY OF THE FLOWERS OF THE SALICACEAE II

MARY JONES FISHER

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POPULUS

Wesmael (15) groups the species of poplars in three tribes which are separated chiefly on the basis of presence or absence of gluten covering the terminal buds, number of stamens, and shape of petioles. Five species were studied, representing the three tribes; *P. alba*, *P. grandidentata*, and *P. tremuloides* the first, *P. deltoides* the second, and *P. candicans* the third.



TEXT FIG. 10. *Populus tremuloides* ♀. Diagrams showing origin, course, and distribution of the vascular supply to the pistillate flower of a typical poplar. *a*, section in median posterior-anterior plane; *b*, bract; *ped*, pedicel; *p*, pistil; *dis*, disk. *b* to *g*, transverse sections of flower at successively higher levels, *A-A*, *B-B*, *C-C*, etc.; *fs*, floral strand; *bb*, bract bundle; *ped*, pedicel; *pb*, vascular supply to pistil; *dis*, disk; *disb*, vascular supply to disk; *o*, ovary; *mcb*, and *cp*, median carpellary and placental bundles.

¹ The first part of this paper appeared in the preceding issue of the JOURNAL.

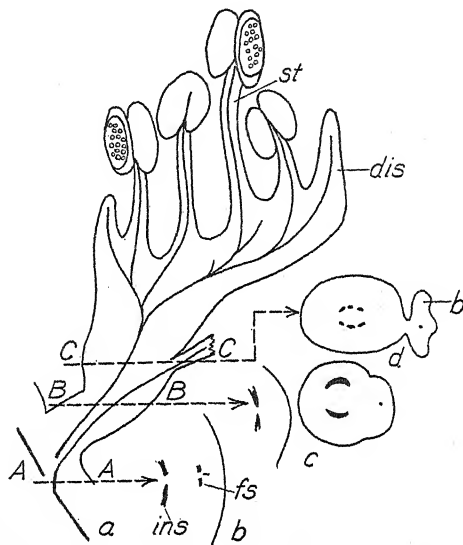
The origin, orientation, and general distribution of the vascular supply to the flowers of species of *Populus* are, with few exceptions, so similar that a detailed description of one or two species is sufficient to present the salient features of normal members of the group.

P. tremuloides ♀ (text fig. 10). The vascular supply to the pistillate flower of this species passes off from the catkin cylinder in the form of a slightly curved segment which is broken almost immediately into two compact strands by the passing out of a single delicate trace from its central region (text fig. 10, *a*, *b*). This trace migrates through the catkin cortex toward the periphery of the floral ridge which is now evident on the side of the catkin, and establishes itself in the tissue which at a higher level is differentiated into the basal part of the lacinate bract. Just above the point where this bract is severed from the floral mass, the trace branches into three bundles which pass through the petiole to the base of the bract-blade where each gives off one or more branches to the incised lobes.

The two remaining strands of the flower supply make their way through the cortex into the protuberance into which the floral ridge has, in the meantime, become transformed, thence through a short "peduncle" (text fig. 10, *a*) into the expanding pedicel of the somewhat trumpet-shaped cup or disk where each strand breaks up into two branches (text fig. 10, *d*). The outer two of the four resultant bundles push upward to the base of the oval cup or disk where, at either end of the long diameter of the oval, each strand forks into two branches. These branches spread apart and in turn bifurcate or trifurcate more or less regularly until the cup is abundantly supplied with vascular tissue (text fig. 10, *f*). The inner two of the four primary strands proliferate and spread open until an oblong siphonostele is formed (text fig. 10, *f*). From this stele additional traces, adaxial and abaxial, also pass off to supply the cup (text fig. 10, *e*), but the major portion of the stele now passes through the center of the pedicellary tissue to the base of the sessile ovary within the subtending cup, where the mode of apportionment of the vascular supply is identical with that of the pistillate form of *Salix alba*. At the base of the ovary the siphonostele spreads out and is finally broken up into four strands, the two smaller of which pass off transversely at either end. These are the dorsal bundles which constitute the midribs of the two fused carpels. The two considerably larger bundles soon pass out to the placentae.

The flower itself may now be considered. At the time of its detachment from the side of the catkin, the floral mass consists of a short axis, at the summit of which is borne the bract. The bract is soon freed and the "peduncle" grades into the tapering base of the inverted cone-like pedicel, where, as just described, the first fragmentation of the two strands occurs. At its summit the peripheral tissues of this pedicellary cone are prolonged to form the obliquely lengthened, cup-shaped disk characteristic of the flowers of *Populus*, the vascular supply to which has already been described.

This cup surrounds the basal half or two-thirds of the conical, two-valved, practically sessile pistil which terminates in two deeply lobed stigmas. The outer and inner tissues of the cup of all species of *Populus* which were investigated are sharply delimited and are readily separated from each other. The inner tissue receives an abundant vascular supply, in contrast with the outer which is entirely destitute of bundles (text fig. 10, g).



TEXT FIG. 11. *Populus tremuloides* ♂. Diagrams showing origin, course, and distribution of the vascular supply to the staminate flower of a typical poplar. *a*, section in median posterior-anterior plane; *st*, stamen; *dis*, disk. *b* to *d*, transverse sections of flower at successively higher levels. *ins*, inflorescence stele; *fs*, floral strand; *b*, bract.

P. tremuloides ♂ (text fig. 11). The origin and distribution of the vascular supply to the sterile flower of this species is very similar to that of the fertile flower. The two strands formed after the departure of the bract trace (text fig. 11, *b*, *c*) maintain their identity through the pedicel, but bifurcate or trifurcate at either end of the long diameter of the oval base of the cup into which the pedicel merges (text fig. 11, *a-d*). The branches spread apart in conformity with the increasingly widening cup and in turn divide and subdivide more or less dichotomously until branches of a third and fourth rank are produced. One fork of each or of the majority of the ultimate subdivisions of these branches becomes the vascular supply to a stamen which is borne upon the cup, whilst the other passes outward, supplying the periphery of the cup itself.

The staminate and the pistillate flowers differ somewhat in shape and structure. In the sterile form the bract is adnate to the pedicel or to the base of the cup, instead of being borne at the summit of a "peduncle" as

in the fertile flower. The cup, upon the sides of which the five to seven stamens are borne, is much more pronouncedly "obliquely lengthened in front" than is the cup of the fertile flower.

P. grandidentata, ♂, ♀, and hermaphrodite (text fig. 11). The vascular supply to typical, normally dioecious flowers of *P. grandidentata* is essentially like that to the flowers of *P. tremuloides*; hence a description may be omitted. The anatomy of perfect flowers, however, needs attention.

Hastings (20) has described "abnormal flowers" of this species from a tree growing on the Palisades, New York. On this tree were found catkins consisting of pistillate flowers only, and also others "made up of staminate, pistillate, and perfect flowers arranged without regular order. On some of the catkins staminate flowers predominated, on others pistillate—but most of them were made up almost entirely of perfect flowers."

In the initial stages the supply to the perfect flower cannot be distinguished from that of the pistillate form. At about the level at which the ovary is freed from the cup in these ambisporangiate flowers, however, there occurs a splitting of a bundle (or of more than one bundle if two or more stamens are to be supplied) which normally supplies the cup. One fork resulting from this dichotomous branching advances toward the periphery of the cup for some distance before it is deflected into the filament of the stamen which is borne on the side of the cup considerably above the base of the ovary.

P. candicans ♂. The origin and general distribution of the vascular supply to the sterile flower of this species are essentially like that described for *P. tremuloides* ♂. The most significant feature in this species is the very evident spiral arrangement of the stamens. A similar spiral arrangement of the stamens in *P. grandidentata* was also observed, although the crowding together of floral parts in the smaller flower of this species makes the spiral somewhat less evident.

The outer margin of the cup in *P. candicans*, as well as that of all staminate flowers of species of poplars studied, is strengthened by a band of compact parenchymatous tissue beyond or within which stamens are never developed, although this tissue itself receives an ample vascular supply.

P. deltoides ♀. The vascular supply to the pistillate form of this species is essentially like that of the fertile flowers of other species of *Populus*, but an increase in number of carpels from typically two, characteristic of the Salicaceae as a whole, to persistently three or four, results, of necessity, in an increase in number of bundles. As the typical dual strand passes through the pedicel and approaches the base of the cup, an oblong, dissected siphonostele is formed. This stele flattens out at the summit of the pedicel and from either end of its long diameter a single trace (sometimes two traces) passes off transversely to the cup, each leaving in its rear a bundle which subsequently becomes a dorsal trace to one of the four carpels. This

is followed very shortly by a similar splitting of strands at the short diameter of the stele during which the two remaining dorsal bundles become differentiated from the vascular supply to the cup. The four dorsal bundles are now established at the apices of a diamond-shaped area and stake out, as it were, the claims of the ovary, the base of which is already foreshadowed. The remaining bundles of the stele have, in the meantime, also gone through a similar process of splitting and apportionment to the cup and ovary during which the placental bundles appear. The vascular supply at this stage is definitely separated into an outer and an inner concentric series of bundles, the former supplying the cup and the latter the ovary. The bundles of the outer series pass off as eight or nine separate strands which fork almost immediately as they migrate toward the periphery of the cup. The inner series comprises the four dorsal bundles, the positions of which have already been indicated, and the vascular supply to the placentae. There is apparently never a complete fusion of the opposed marginal strands which together comprise the supply to a single placenta. Branches from the placental bundles supply the thick-walled ovary as well as the ovules. Each of the four dorsal bundles passes up through the walls of the ovary to the base of the short style where it splits into two which supply one of the four stigma lobes.

DISCUSSION

From the foregoing description it is apparent that species within each genus can be arranged in an ascending series illustrating an orderly progression, based upon generally accepted principles of evolution, from lower to higher rank. This progression is, as just stated, more marked among the staminate flowers of *Salix*, where the pleiandrous willow, *S. safsaf*, marks one extreme, and the diandrous form, *S. purpurea*, the other. The comparatively numerous microsporophylls, together with the fact that these stamens are spirally arranged on an elongate axis, are indicative of the relatively primitive estate of *S. safsaf*; and, conversely, the extreme reduction in number of sporophylls and the arrangement of these stamens in a single whorl on a short axis proclaim the more evolved condition of *S. purpurea*.

The first step toward bridging the gap between these two extremes takes place in the pleiandrous group, where in both *S. capensis* and *S. Humboldtiana* (tropical or subtropical forms) there is not only a reduction and tendency toward stabilization in number of stamens, but the primitive spiral arrangement of these organs is, in the latter species, in process of being discarded for the cyclic or more advanced type.

There is further reduction, but less constancy, in number of stamens in the north-temperate forms, *S. amygdaloides* and *S. nigra*. There are indications, however, that three is becoming the dominant number in each of these species. In *S. Bonplandiana* three stamens are definitely estab-

lished. A relatively recent transition from the primitive pleiandrous to the advanced diandrous estate is illustrated by *S. alba* and *S. babylonica*, each of which is included in the former group by Andersson, and in the latter by Gray.

The nectaries, which range in number from many and indefinite in the staminate flowers of the pleiandrous willows to definitely one in the pistillate form of the pleiandrous group, and one and two in both staminate and pistillate flowers of species of the diandrous group, also form, though to a lesser degree, an ascending sequence similar to that of the stamens described above. These structures, the morphology of which will be discussed later, together with the stamens, are illustrative of the fact that evolutionary tendencies among members of different floral sets in a single flower, or, indeed, among homologous structures in pistillate and staminate flowers of the same species, are not necessarily expressed simultaneously. Thus, whereas the stamens in flowers from species near the summit of the pleiandrous line have become definitely fixed in number, the nectaries remain inconstant in number throughout this group in the staminate flowers. The nectaries, on the contrary, show an advance over the stamens of these same species in that (with the possible exception of *S. safsaf*) they are, even in the most primitive forms, always definitely arranged in one or two whorls instead of in spirals.

Floral progression in species of poplars is, doubtless because of the small number of living species, not so evident as in the willows. There is, however, a considerable reduction in number of stamens, from over sixty in the staminate flower of *P. deltoides* to eight or nine in *P. grandidentata*.

Progressive stages through which the now practically stabilized gynoeceum of both *Salix* and *Populus* may have passed in attaining the present highly evolved type of pistil with its two fused carpels and unilocular ovary, are suggested by the fertile flowers of *P. deltoides* in which the pistils as a rule consist of three or four fused carpels.

Nectary of *Salix*

The indiscriminate application of the term nectary to any organs which secrete nectar, no matter how different these may be morphologically, is reflected in the variance of opinion expressed in literature on the subject. A compilation of historical data reveals the fact that so-called nectaries fall naturally into two broad, unrelated groups of structures: (1) those which are mere glands, disks, emergences, or localized areas of tissue the function of which is primarily that of secreting honey or nectar; and (2) those which represent reduced or remodelled organs which have secondarily assumed the secretory function. The former type has never possessed a vascular supply whereas the latter usually retains bundles or vestigial elements reminiscent of former vascular tissue.

The morphology of the nectary, which is always present in flowers of

Salix, has given rise to diverse theories in regard to the phyletic history of this structure which is practically unique among the so-called Amentiferae. Investigators have usually assigned the nectary to one of the four following categories: (a) emergences or glands called into existence in response to an entomophilous mode of pollination; (b) modified and reduced sporophylls; (c) modified bracts or bracteoles; (d) reduced perianth parts.

The first in the above list represents, apparently, the current view entertained by writers in the latter half of the past century. Eichler, 1878 (who does not himself, however, accept this interpretation), states that most authors recognize in these glands the accessory characters of emergences only. Henslow (21) 1880, says, "Authors regard the little prominence at the base of the stamens and of the pistil in *Salix* either as the calyx or else as the axis. It appears to me to be simply a cellular gland for secreting honey, which they do abundantly. I could detect no spiral vessels in them at all." Chamberlain (13) 1898, states that "there is nothing in its [the nectary's] history which would allow it to be regarded as a reduced or transformed floral organ."

The second view—that these glands are reduced sporophylls—finds frequent expression in botanical literature, and accords with the opinion of Prantl who held that nectaries in flowers in general have been transformed from the outermost stamens. The third is supported, among others, by Velenovsky (32) who homologizes the adaxial gland, which in *S. aurita* (the species under observation) tends to split into two practically equal portions which then shift from a median to lateral positions, with the adaxially placed bracteoles of the flower of *Juglans regia*. He has, however, a different interpretation for the abaxial gland which he regards as homologous with the two or three "perigons" of the walnut flower.

The dictum of manuals of botany in general, that the flowers of Salicaceae are "without perianth," has been, for the most part, so unquestionably accepted that a fourth possible interpretation of the nectary in the flower of *Salix* has rarely been suggested. Bentham and Hooker, however, are among the few who have questioned whether the disk of *Populus* and the nectary of *Salix* may not in reality be of perianth nature; and it has also been pointed out recently (2) that the conspicuous structure below the stamens and pistil of the ambisporangiate flower of *P. glauca* Haines (a poplar native to India, named and described by Haines (18) in 1906), is "undoubted perianth." This perianth, in turn, has been tentatively homologized by some writers with the nectary of the willow flower.

In the light of the above citations, we may now weigh the evidence obtained from an investigation of over thirty species of *Salix* to ascertain the nature of the nectary.

The repeated occurrence of from one to three vascular bundles or vestiges of bundles within the tissues of the nectary of many species (*S. alaxensis* ♀, *S. amygdaloides* ♀, *S. cordata* ♀, *S. orbicularis* ♀, *S. reticulata*

♂, *S. tenuis* ♂ and ♀, *S. tristis* ♀, *S. uva-ursi* ♀, *S. vestita* ♀) leaves no doubt whatever but that these structures formerly received an ample vascular supply, which in some species has now disappeared entirely, while in others the bundles are still in process of fading out. In some species a reduction of the vascular supply to the nectary has taken place in such a manner that only isolated vestiges of strands, which now have no connection whatever with any other vascular tissue, are left to mark the site of the vanishing bundle or bundles. In others, straggling remnants originating from the periphery of the floral stele pass out toward the nectary but fade out before actually reaching that organ. In still others (*S. alaxensis* ♀, *S. Bonplandiana* ♀) the entire course of the vascular supply can be definitely traced from the time of its origin from the floral stele to its ultimate establishment within the tissues of the nectary itself. The frequent presence of vascular bundles in these so-called nectaries is sufficient in itself to warrant the conviction that these structures are true organs and would, therefore, be incorrectly placed in the first category.

It has been pointed out by Mrs. Arber (1) that "when evolution proceeds in the direction of reduction, vascular structure generally, though not always, lags behind the outward form, and becomes diminished to a vanishing point at a less rapid rate than the surface features. In other words, when an organ is becoming rudimentary and is on the point of disappearing, the branches of vascular tissue which formerly supplied it are generally still traceable, though they may be reduced to mere stumps."

Conclusive evidence that these structures are not mere glands, as well as that their primitive function was not that of secreting honey, is most strikingly afforded by the primitive, tropical species, *S. Bonplandiana*, *S. capensis*, and *S. safsaf*, the glands of which are not only non-nectariferous in character, but are also expanded and petaloid.

Any structures of doubtful affinity which appear in the vicinity of microsporophylls are frequently designated "reduced stamens." It is not surprising, therefore, that the organs found at the base of flowers of *Salix* should be confidently interpreted by some writers as reduced stamens. The occasional appearance of nectaries with superficial resemblance to stamens, together with the suggestive proximity of the nectaries to the outermost stamens, serves as evidence that these glands may be reduced stamens. If this be so, we should expect, incident upon the reduction of sporophylls from many to few (two), an inverse ratio in number of stamens and nectaries present in the flower. This, however, is not the case, for, as we have seen, there is apparently a synchronous reduction in numbers of the two sets of organs. Furthermore, if nectaries are only reduced stamens, their position upon the floral axis should not only coincide with positions formerly occupied by the unmodified stamens, but their transition from spiral to cyclic arrangement should also follow and reflect successive stages in the phyletic history of the androecium. On the contrary, the

cyclic arrangement of the nectaries is definitely established in flowers in which the primitive spiral arrangement of stamens still obtains.

Despite the remarkable diversity of form exhibited by the nectary of *Salix*, there is a manifest tendency, especially pronounced among species of the diandrous group, and also suggested in the three-notched, cup-shaped gland of the pistillate flowers of some species of the pleiandrous group (*S. Bonplandiana*, *S. safsaf*), for these organs to be more or less deeply bi- or trilobed at their distal ends, a condition surely not suggestive of stamen-nature.

A microscopic investigation of the internal anatomy of the nectary reveals, as we have just seen, evidence sufficient to justify the conclusion that these structures were originally supplied by three main vascular bundles. The stamen, on the other hand, is almost invariably supplied by a single vascular strand. If the nectaries are reduced stamens, the numerical difference between the vascular supply to the two sets of organs could be accounted for only by assuming that the nectary represents a number of microsporophylls, which have been fused in groups of two's or three's.

In both staminate and pistillate flowers the vascular supply to the nectary, whether persisting in its entirety or represented merely by vestigial strands which project from the adaxial side of the stele, invariably passes off from the floral stele well in advance of the bundles which are subsequently given off to the stamens or carpels.

Cumulative evidence that the nectaries of *Salix* cannot be correctly interpreted as reduced or transformed sporophylls may, then, be briefly summarized: (a) The attainment by the nectary or nectaries of the more advanced cyclic arrangement in flowers in which the stamens still adhere to the primitive spiral mode; (b) the vascular supply of three bundles to the nectary in contrast with the single bundle which supplies the stamen; (c) the origin of the vascular supply to the nectary from the floral stele at a considerably lower level than the strands to the sporophylls; and (d) an entire lack of teratological evidence that nectaries are sporophylls.

Two other possibilities remain: (1) that these structures are modified bracts or bracteoles, or (2) that they represent reduced perianth parts. A rigid delimiting of bracts (which, in the willows, are unquestionably leaves) from perianth parts is, obviously, a hazardous undertaking when it is recalled that "sepals and petals may be regarded as often leaves more or less modified to serve as floral envelopes, and are not so different from leaves in structure and function as to deserve a separate morphological category."

The vascular supply to the nectary agrees in number and in position of bundles with the strands which supply the typical bract. The three bundles to the bract, however, pass off from the floral stele at a lower level and considerably in advance of the three bundles which are given off to

the nectary. The two sets of organs are, therefore, evidently not members of the same series or cycle. The lobing at the distal end of the nectaries does not find a counterpart in the bract of *Salix*. In fact, "scales entire" is so constant a feature of the bract of the willows that systematists make use of this character as a basis, primarily, for separating *Salix* and *Populus*.

The nectaries, as we have seen, have attained the cyclic mode of arrangement and are intercalated between the bract and the sporophylls in one, or apparently sometimes two whorls, the component parts of which seemingly alternate with the sporophylls.

These organs, differing also in this respect from the bracts, display remarkable diversity of form, ranging from conspicuous flat, non-nectariferous, petaloid structures in the staminate species of the pleiandrous group (text fig. 8, *e*), to inconspicuous, fleshy, nectariferous glands in many species of the diandrous group. This variation in form of the gland is paralleled by a similar diversity in the extent to which reduction or fusion of parts of the nectary may have taken place. In the pistillate form of species of the pleiandrous group, *S. Bonplandiana*, *S. safsaf* (text figs. 1, *d*; 9, *f*, *g*), a single cup-like nectary entirely surrounds the stipe of the ovary; a single nectary is also found in the pistillate flowers of the majority of the diandrous forms, but the single nectary is here reduced to a relatively small, adaxially-placed gland.

An interesting series showing different degrees of fusion of parts of the nectary may be found frequently in flowers of the same species as, for instance, in *S. safsaf* (text fig. 8, *d*, *e*) in which the nectary of the staminate flowers varies from a bilabiate, corolla-like structure, the proximal end of which is a tube surrounding the sporophylls, to a whorl consisting of a varying number of unfused parts. Between these extremes may be placed those thin, broad, petal-like structures which are attached to the floral axis by several claws (text fig. 8, *e*). This mode of attachment suggests that a lateral fusion, or an incomplete separation of members of the nectary whorl, has occurred.

In some of the more primitive species of *Salix* in which the nectaries are non-nectariferous (*S. capensis*), the abaxial series of glands are larger and more conspicuous than those on the adaxial side, which is just the reverse of conditions obtaining in the more highly evolved species, where, indeed, the abaxial glands are frequently lacking. With the assumption of the entomophilous mode of pollination there has been, apparently, a reversal in size and importance of the two groups of nectaries. This may be accounted for, possibly, by the fact that sporophylls show a marked tendency to droop in such a manner as to conceal the abaxial glands, while at the same time exposing the adaxial nectaries and affording a comfortable foothold for the insect. The weight of the insect body doubtless presses the sporophylls more firmly against the abaxial nectaries, in consequence rendering these glands still less accessible, with the result, following the

inevitable law of disuse of a member, that these organs are dropping out from the nectary cycle.

The question now arises as to what conclusions can reasonably be drawn, from the evidence obtained, in regard to the morphological nature of the nectary. The nectary, as we have seen, is bract-like so far as the number and mode of distribution of the bundles which compose its vascular supply is concerned. But the origin of these bundles at a higher level from the floral stele discloses the fact that the nectaries and bract do not belong to the same series. This alone, however, is not sufficient to warrant the conclusion that nectaries are not, therefore, morphologically bracts, since the appearance of one or more series of bracts in acropetal succession is not an uncommon occurrence in flowers of many angiospermous groups.

The persistent lobing of the nectary is probably the retention of a feature which is reminiscent of a bi- or trilobed ancestral structure. The remarkable diversity in both form and number of nectaries displayed by species within the genus may be accounted for, possibly, on the grounds that these organs represent old structures which have been lately re modelled to subserve a recently acquired function. The bract, in contrast, is never lobed, is invariable in form and number, and is evidently an organ of unquestioned morphological individuality with permanently established characters.

With the exception of a similarity in number and mode of distribution of its bundles, the nectary has, apparently, little in common with the bract. The level at which these bundles arise from the floral stele affords corroborative evidence that the nectaries, morphologically as well as apparently, are structures which are inserted in one or more whorls between the bract below and the sporophylls above. This position of the nectaries on the floral axis, the lobing, the frequently relatively broad and petaloid appearance, suggestively associated in the more primitive willows with "nectaries" which are non-nectariferous in character, seemingly affords a significant clew to the past history of these structures, and leads to the not unnatural conclusion that nectaries are reduced perianth parts, which were formerly lobed and more or less fused. Whether these parts represent calyx or corolla, or calyx plus corolla (there is evidence in some species of two whorls, one above the other), cannot now be ascertained. The condition in some of the primitive species suggests a possible gamophyllous perianth which may have been even irregular or bilabiate. There is also in the number and arrangement of traces a suggestion that the perianth parts were on the plan of 3 or 6.

Sporophylls

In all species examined, the stamens and pistils in normal flowers of both *Salix* and *Populus* (with the single exception of *P. deltoides*) have attained stability of form and structure. Each stamen is invariably supplied

with a single vascular bundle; each pistil, consisting of two united carpels, receives invariably six bundles, the ventral (marginal) ones of which are usually fused into two large placental bundles. Fusion in the gynoecium has gone so far that a unilocular ovary is formed.

An exception to this definite bicarpellary type of ovary, characteristic of the fertile flowers of the Salicaceae as a whole, is found in *P. deltoides*, the gynoecium of which consists of occasionally two, but more frequently of three or four carpels. These carpels always receive the normal supply of vascular bundles, and also form through their fusion the typical unilocular ovary. In the matter of reduction of sporophylls, therefore, *P. deltoides* has lagged behind, and on this basis may be regarded the most primitive member of the family among the species studied.

Bracts of the Salicaceae

The foliar nature of the bract is clearly manifest by the nature of its vascular supply which is similar to that of a normal leaf, and which, in its origin, causes the usual gaps; by the retention in primitive species of well-developed stipules (text fig. II, *b*); and by the frequent presence, when stipules are absent, of stipular traces. It is apparently not, however, the leaf in the axil of which the flower arises; were this the condition its traces would arise from the stele of the catkin below the point of origin of the supply to the flower. Since the traces, however, are invariably derived from the floral stele, the bract must clearly be interpreted as appertaining to the flower, and situated upon a lateral branch.

The retention by this small, ephemeral bract of the three traces characteristic of the willow leaf and also of stipular traces when stipules have disappeared, is a remarkable instance of the retention by leaf traces of ancestral conditions.

In the diandrous forms this bract presents the appearance of a leaf subtending a sessile flower; in the more primitive, pleiandrous willows it appears as a leaf at the base of the pedicel, which arises from a short, thick, stalk of peculiar structure. This stalk seems to represent a branch of the inflorescence—a lateral stem upon which the flower of the ancestral forms was borne. Whether this branch bore one or more leaves and one or more flowers it is now impossible to state. (See also the discussion of the inflorescence, below.)

Although the vascular supply to the bract is always derived from the stele of the flower, there is, nevertheless, a striking and undoubtedly significant difference between the number of strands which are primarily given off to the bract of some of the more primitive tropical forms (*S. Bonplandiana* ♀, *S. capensis* ♂, *S. Humboldtiana* ♂ and ♀) and the number which pass to the bract of some of the more highly evolved species. In the tropical forms just cited, strong median traces, or else a single median trace which bifurcates or trifurcates almost immediately, pass out, leaving

a single gap in the floral stele. In the higher types, three traces pass off more or less simultaneously from three different regions of the stele, each (if the stele be a complete cylinder) leaving a gap to mark its place of exit.

The bract of *Populus*, like that of *Salix*, is clearly foliar in nature and its vascular supply is likewise always derived from the stele of the flower. In respect to the bract traces there is a striking similarity between *Populus* and the more primitive willows cited above. In all species of *Populus* examined the vascular supply to the bract invariably passes off from the floral stele as a single strand leaving a single gap.

Sinnott (30) states that it is generally recognized that during the course of evolution among vascular plants certain organs or regions of the plant-body have changed more slowly than others and hence have retained many ancient characters which have been lost elsewhere. Among these organs or plant parts the anatomy of the leaf, more especially the node where the leaf and stem unite, often retains very strikingly features which have been lost elsewhere in the plant. He is of the opinion that "a foliar supply of three bundles, each causing a leaf gap of its own in the stem cylinder, is certainly a very ancient type among the dicotyledons." This he designates the "trilacunar condition;" a condition which is characteristic of most of the "Amentiferae" (including Salicales) and is present in the majority of the Ranales and Rosales. This trilacunar condition, according to Sinnott, has been modified by reduction into a single-gap or "unilacunar type of nodal structure" either through approximation of the three gaps and their coalescence into one, or through the disappearance of the two lateral bundles and gaps. From the apparently derived condition of the unilacunar type, together with the predominance of the trilacunar condition in various families of the lower Archichlamydeae and "especially in the presumably primitive Amentiferae" the "trilacunar type is regarded as the most ancient angiosperm condition." He further states that "in all species of *Salix* and *Populus* examined, the nodal structure was invariably trilacunar."

Were the present investigations limited to species of *Salix* from the arctic and temperate regions only, the evidence obtained would substantiate, in a measure, the preceding theory regarding the relative primitiveness of the tri- and unilacunar types of nodal structure, since the occasional occurrence of the unilacunar condition (*S. amplifolia*, *S. arctica*, etc.) among these predominantly trilacunar types could then have been interpreted as an advanced condition. The obvious difficulty of forcing the clearly primitive genus *Populus*, the nodal structure of the bract of which is invariably of the unilacunar type, into the same category, may, of course, be surmounted by recalling the fact that evolutionary tendencies are not necessarily expressed simultaneously either among genera of the same family, or, indeed, among floral members of different whorls of even the same flower. The more primitive nature of the poplar flower in general does not readily accord, however, with such an interpretation.

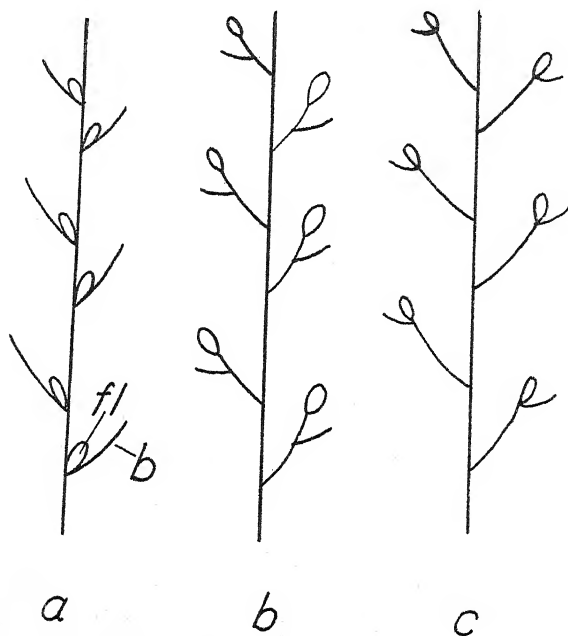
The extreme isolation of a few species of pleiandrous tropical willows (notably *S. Humboldtiana*) affords an illustration of the principle formulated by Berry (9), that for geological time as a whole climates have been more uniform than at present. For these species of *Salix* are survivors and undoubtedly direct descendants of ancestors of more extensive range which flourished under an equable climate characteristic of the Cretaceous and early Tertiary when there were no polar, temperate, or equatorial zones, and the same plants were found near the poles and at the equator. Since climatic conditions in the tropics appear to have remained essentially the same throughout recent geological periods, we should naturally expect to find a retention of practically unmodified ancestral features among those plants which have not been obliged to adapt themselves to totally new conditions and habitats. It may be, therefore, of significance that the bract of these more primitive tropical willows displays, in common with the bracts of *Populus*, the generally conceded more primitive genus, the unilacunar type of nodal structure. Thus the unilacunar type may be for the Salicaceae the more ancient. But it is, however, fully as likely that the more primitive groups of this family have progressed further in the modification of the bract supply than have the more advanced types.

Inflorescence

The fact that the flowers of the Salicaceae, especially in the pistillate aments, are not invariably sessile, is recognized by taxonomists. But this non-sessile condition is usually attributed to an elongation of the "pedicels" which are, in fact, morphologically the bases of the stipitate ovaries and not true pedicels. That the true floral receptacle is the region of nectary attachment is evident in staminate flowers, and clearly demonstrated to be such by anatomical investigation in pistillate flowers. The flowers of the Salicaceae are, nevertheless, not strictly sessile and their actual pedicellate condition becomes more apparent when species are arranged in a series extending from the more highly specialized diandrous species of *Salix* to the more primitive pleiandrous forms, among which may be included those of *Populus*.

In the diandrous group reduction has gone so far that the pseudo-sessile nature of the floral units comprising the inflorescence is revealed only through microscopic investigation. That the bract, though superficially appearing to belong to the catkin and to subtend the flower, is actually an appendage of the floral branch itself, is clearly evident from the source of origin of its vascular supply which, as we have seen, is invariably from the floral stele. The appended diagrams depict apparent (text fig. 12, *a*), and actual (text fig. 12, *b*, *c*) conditions existing apparently throughout the Salicaceae. The presence of the reduced branch between the flower and the axis of the catkin is hardly to be detected in the more highly specialized diandrous willows where extreme reduction has taken place, and might not

be recognized. In the pleiandrous willows, on the other hand, as well as in the poplars, a persistent, stubby internode (peduncle and pedicel?), upon which are borne the bract and flower, is still microscopically apparent, and affords the clue to the path along which internodal suppression has un-



TEXT FIG. 12. Diagrams depicting *apparent* (a), and *actual* (b, c) conditions obtaining in salicean inflorescences, apparently throughout the group. *fl*, flower; *b*, bract.

doubtedly taken place. This external evidence of the previous existence of one, or possibly more, internodes between the flower and the axis of the ament is likewise confirmed by the anatomical structure of both parenchymatous and vascular tissues. The former presents a telescoped appearance (*S. Humboldtiana* ♀, text fig. 9, *d*) such as might easily be brought about through a process of shortening and compressing of internodes and pedicels of floral units of a raceme, or of a raceme-like type of inflorescence; the vascular tissue passes through this compact parenchyma as a single strand just as the stele traverses a typical elongate branch or internode.

In the more highly specialized species of *Salix* this reduction and suppression of the internode has culminated in the production of a compact ament the flowers of which are to all outward appearances sessile (text fig. 12, *a*).

Parkin (26) advances the theory that all flower groupings may be traced to one primitive source, *viz.* a leafy shoot bearing a solitary flower.

From such a shoot, simple cymose flower-clusters are shown to arise first, and from these in turn are evolved racemose forms. By suppression of the internodes of the inflorescence, either of the lateral or main axis or both, the different varieties of racemose inflorescences (spike, umbel, head, etc.) can be derived from the raceme. The raceme or spike originating thus is consequently by no means an early form of inflorescence but a highly specialized one which has had a cymose ancestry.

It is clearly evident that the inflorescence of the Salicaceae has undergone considerable reduction in attaining its present structure, and it is probable that the earlier steps in this process are now entirely obliterated. Whether the primitive inflorescence was cymose, or whether each flower as it exists to-day is a pseudo-axillary flower which alone has survived an extreme reduction of peduncle, bracts, and flowers of a former axillary inflorescence (with a history similar to that of the solitary axillary flowers of certain species of shrubby Papilionaceae described by Parkin), cannot be definitely ascertained. On the other hand there is afforded by the pleiandrous species of *Salix* and by *Populus* a superabundance of evidence of a transitional stage from an already comparatively advanced raceme to the still higher spike (ament) which, together with the capitulum of the Compositae, represents the apogee of inflorescence evolution.

CONCLUSIONS

Before definite conclusions can be drawn in regard to the phyletic rank of any plant group, it is necessary that all testimony be in. This, as pointed out by Coulter (14), involves a comprehensive knowledge of the history, morphology, and vascular anatomy of the group.

The anatomy of the salicean flower has already been described in considerable detail. Investigation by Miss Holden (24) reveals the fact that in wood structure the Salicaceae are also of high rank. We may, therefore, now turn to history for further evidence of the phylogenetic level of the family. Penhallow (28) states that "among forty-five families of angiosperms now known to constitute the flora of the Cretaceous and Tertiary ages in North America, it will be found that, geologically speaking, the Salicaceae is by no means the primitive group which seems to be implied by the position usually assigned it, but that it really occupies a position which is twenty-fourth in a series based upon the percentage ratio of occurrence in the two great geological periods." He further adds there is thus a "very strong suggestion (1) that the Salicaceae is in no sense a primitive family from the standpoint of geological succession, and (2), it is, as a whole, much more characteristic of the Tertiary than of the Cretaceous." In other words it is a family which seems to have been in the process of development in the Tertiary, hence is, in its present condition, of recent origin. He believes also that in Cretaceous times the family was compatible with a much warmer climate than at present as evinced by the survival of tropical forms to-day.

Morphology and anatomy are so closely allied that in the foregoing anatomical discussion morphology, at least in its broader aspects, has been included. To the more salient features certain minor ones may be added as affording cumulative evidence of the relatively high position of the Salicaceae, such as the presence of anatropous ovules instead of the more primitive orthotropous type, and the possession of seeds without endosperm which are generally conceded to be derived and higher than endospermous seeds.

The predominantly entomophilous habit of *Salix*—a most striking and unusual occurrence among the Amentiferae—in contrast to the universally anemophilous mode of pollination of *Populus*, has given rise to a marked divergence of opinion as to whether this be a primitive or a derived feature. Henslow (22) thinks “there is little doubt but that all wind-fertilized angiosperms are degradations from insect-fertilized flowers.” He believes, however, that pollination by insects may be recovered in anemophilous flowers when honey may again be secreted, and cites *Salix caprea* as an example. Arber and Parkin (2) are of the opinion that the entomophily of *Salix* is a recently acquired character; at the same time they regard *Populus* itself as not primitively anemophilous, but derived originally from entomophilous ancestors. They suggest that “the re-adoption of entomophily has possibly been the saving of the willows.”

If these views be accepted, it is obvious that the entomophily of *Salix* of to-day has evolved far from the entomophilous habit of the primitive salicean ancestor, since this genus has thus passed successively from entomophily through anemophily and thence back in its highest forms to entomophily again. *Populus*, on the other hand, has progressed only so far as the second stage and has stopped with the acquirement of the apparently fixed anemophilous habit.

For evidence supporting these theories we again turn to surviving descendants of the Salicaceae of the Cretaceous and Tertiary times which are living to-day under practically the same climatic conditions as those under which the family is believed to have originated. It is, therefore, exceedingly suggestive that species of tropical willows (*S. safsaf*, *S. capensis*), have many and indefinite stamens and relatively large, petaloid (or, in the corresponding pistillate forms, cup-shaped) nectaries, non-nectariferous in nature. These primitive forms, like the poplars with which they show close affinity in other structural and anatomical features, have clearly attained anemophily from a primitive entomophily. That the anemophilous habit in the less specialized species of *Salix* may possibly be more widespread than is generally recognized is suggested by the fact that Warming regards the willows of Greenland as anemophilous.

The gap between anemophily and entomophily is apparently bridged by certain species of pleiandrous willows (*S. amygdaloides*, *S. nigra*, etc.) of the temperate regions. In these forms a reduction in number of stamens

and hence of pollen is evidently taking place. These losses, however, are offset by the development of nectar-secreting structures—the adaptation of old structures to a new function, the transformation of perianth vestiges into nectaries.

The tropical and sub-tropical willows are clearly the most primitive and the arctic species follow these closely in the structure of the degenerate perianth and in the presence of anemophily. The temperate climate species are the most advanced.

The theory that the primitive angiospermous flower was amphisporangiate with indefinite macro- and microsporophylls, below which a well-marked perianth subserved the double function of protection and of “playing some part in the mechanism for insuring cross fertilization” has been propounded by Arber and Parkin, Goebel, Henslow, Hallier, and many others. It is not possible here to enter into arguments for or against this view which appears to be tenable.

Assuming that the ancestral flower of the Salicaceae possessed a well-developed perianth of several more or less similar members, perhaps with an inner series which served both as an attractive and a protective structure, it is not difficult to interpret the nectaries of *Salix*, which are unquestionably reduced organs, as unobtrusive representatives of conspicuous petaloid ancestors correlated with primitive entomophily. Despite the relatively archaic features obtaining in the poplars and the pleiandrous willows of the tropics, it is, nevertheless, a far cry from such a perianth to the cup-like, frequently three-notched gamophyllous “nectary” of the pistillate form of the latter, or to the gamophyllous “cup-shaped disk” of the former. It is of course a much longer way to the single, inconspicuous nectary of the diandrous willows.

The antiquity of the amphisporangiate flower as compared with the simple monosporangiate type is strongly supported by Arber and Parkin who conclude from a general survey of existing angiosperms that “the Apetalous orders without perianth, such as Piperales, Amentiferous families, and Pandanales, cannot be regarded as primitive Angiosperms,” thus dissenting from the view advocated by Engler especially and accepted by many modern botanists, that the primitive angiospermous flower is to be sought among monosporangiate, apetalous forms. Henslow (22) also regards the amphisporangiate condition as primitive and points out that many instances exist of the same species having male, female, and hermaphrodite flowers.

The lack of traces indicative of amphisporangiate ancestry in normal flowers of the more highly specialized Salicaceae further suggests the remote period at which reduction of these floral structures must have occurred and emphasizes further the specialization of this family. Even among the more primitive willows and poplars such traces are of rare occurrence in so-called normal flowers. During the present study which involved investi-

gation of innumerable staminate and pistillate flowers from almost forty different species, only two cases (apart from the perfect flowers of *Populus grandidentata* described) were noted in which vestigial traces in monosporangiate flowers were interpreted as harking back to an amphisporangiate ancestry. These exceptional cases occur in the pistillate flower of *S. Humboldtiana* (text fig. 9, e) where there are apparently present remnants of stamens, and in some staminate flowers of *Populus grandidentata* where evidence of a pistil was found.

Further, if teratological evidence is illustrative of the fact that "any diclinous plant may reproduce by reversion the lost sex," the theory of the amphisporangiate ancestry of the Salicaceae must needs be accepted owing to the frequency in which perfect flowers make their appearance in even the most highly evolved species of Salicaceae. To this must be added the evidence afforded by the nine trees of *Populus glauca* Haines with entirely "2-sexual flowers." The perfect flowers of *P. grandidentata*, the vascular supply to which presents no abnormal features, likewise point to an amphisporangiate ancestry.

Relative Primitiveness of the Two Genera, and Phyletic Rank of the Family

Measured by generally accepted botanical dicta, and substantiated by historical record, *Populus* is undoubtedly the more primitive of the two genera. This is aptly summarized by Penhallow who states: "General trend of the evidence so far collected—geographical, geological, anatomical—is all in one direction, and that is to show that the genus *Populus* is essentially the more primitive member of the family, and that it is the genus through which we must probably seek connection with ancestral forms." The close relationship existing between the poplars and primitive willows has already been considered.

The origin of the Salicaceae, like that of the angiospermous group as a whole, remains an unsolved mystery; and, indeed, we can point with confidence to no family as closely allied, although a number of authors (including Bessey (10) who agrees with Nidenzu, in Engler and Prantl, in thinking the Salicaceae show affinity with the Tamaricaceae) have called attention to the resemblance between the flowers of the Salicaceae and the tamarisks. Robertson (29) likewise is not averse to this view and states: "For the relations of Salicaceae I would look among the entomophilous polyspermous forms having similar fruit and seed. So from my standpoint, and in the absence of some other considerations, I would readily accept the view that the Salicaceae should follow the Tamaricaceae, the reduction of flowers being correlated with close crowding in a specialized inflorescence." On the other hand Pax, in Engler and Prantl, among others, is of the opinion that the Salicaceae have nothing significant in common with the Tamaricaceae.

Apropos of this search after affinities, Wernham (33) has wisely pointed

out that "it is all-important that no consideration should be left unnoticed before conclusion is arrived at; and too much stress cannot be laid upon that most obvious, most general, and yet so often-neglected principle that *presumption of affinity should not be based upon a single character; the characters employed as criteria should be as numerous and as distinctive as possible*; and biological evidence should be carefully examined in the light of history in the descent of the plant-forms under investigation."

An anatomical investigation of species of *Tamarix*, the results of which the writer hopes to give in a subsequent paper, tends to confirm the view held by Pax that the resemblance between flowers of the two groups is merely an interesting case of parallelism in grosser features which is not reflected in the fine anatomy, more especially in the distribution of the vascular supply.

Whilst the origin of the Salicaceae is apparently wrapped in obscurity, and the affinities of the group at present purely conjectural, there is overwhelming evidence of extreme reduction in flower and inflorescence which proclaims the highly evolved position of the family. The misleading simplicity of the flower is due in large part to reduction and not to primitiveness, and the group should be, therefore, accorded a phyletic rank far above that to which it is usually assigned by taxonomists.

METHODS

The flowers for this study were killed in chrom-acetic acid, and imbedded in paraffin in the usual way. In cases where herbarium material alone was available, good results were obtained by boiling the dried specimens in a weak solution of potassium hydroxid, then imbedding in paraffin, as with fresh material. Such material proved nearly as satisfactory as living. Light green and safranin proved a satisfactory stain and during the later part of the investigation was used entirely.

SUMMARY

The more important results of this study, which involved macroscopic and microscopic investigation of thirty-seven species of the Salicaceae, may be stated as follows:

1. The simplicity of the flowers of the Salicaceae is largely due to extreme reduction and not to a retention of archaic features. This is shown by:

(a) Proof that these flowers formerly possessed a perianth of several parts, perhaps of two whorls.

(b) The vestigial nature of the nectaries, which represent perianth parts in reduced form and modified function.

(c) The presence of vestigial vascular supply to organs now greatly reduced or entirely lacking.

(d) Evidence that the small number of sporophylls is the result of

reduction within the family; in stamens from several to two, the two in the highest forms fused; in carpels from four to two (in *Populus*), and the attainment in both genera of a specialized syncarpous ovary. Accompanying this change is one from spiral to cyclic stamens.

(e) The evident reduction within the genus *Salix* from an indefinite number of somewhat petaloid, non-nectariferous nectaries to a single, inconspicuous, gland-like structure.

2. The nectaries are surviving, much reduced remnants of a primitive ancestral perianth. The members of this perianth were petaloid and possessed the vascular supply of leaves. In the primitive, tropical, pleiandrous willows the perianth is still more or less petaloid, and is not nectariferous. It is more or less gamophyllous and suggests an irregular, somewhat bilabiate condition. In the highest willows the perianth has been nearly lost, being represented by a single small nectary. Vascular traces to other nectaries (perianth parts) still exist, however.

3. The secretory function of the nectary is secondarily acquired and is correlated with reduction and with reversion to entomophily. The ancient Salicaceae were entomophilous, but became anemophilous. A return to entomophily is now taking place, but only part of the genus *Salix* has attained this advance; *Populus* and the pleiandrous willows are still anemophilous. The diandrous willows, especially arctic species, are often weakly entomophilous.

4. The bract does not subtend the flower, an axial structure on the catkin, but is a leaf upon the pedicel of a formerly stalked ancient flower.

5. The catkin represents a very advanced type of inflorescence, paralleled by the capitulum of the Compositae; it represents a reduced inflorescence in which lateral branches of unknown former extent have been compressed and reduced so as to appear lacking.

6. *Populus* is the more primitive member of the family, the gap between the two genera being bridged by certain species of tropical and southern-hemisphere willows which display intermediate structural and anatomical features.

7. The disk-shaped perianth of *Populus*, or its peripheral parts, is homologous with the nectary of *Salix*.

8. The flowers and reproductive axis of the Salicaceae afford an unusually fine example of the retention, in vascular structure, of ancestral features. This is especially true of the perianth parts and of the traces of the floral bracts, including stipular traces.

9. Morphology and geological record corroborate and sustain the evidence afforded by anatomy, including that of the wood, that the Salicaceae is a highly evolved instead of a primitive family and should, therefore, occupy a position higher than that usually accorded it by taxonomists. Lack of convincing evidence of close alliance with any other family makes it difficult to assign the natural position of the Salicaceae.

The writer wishes to acknowledge her gratitude and indebtedness to Professor A. J. Eames, who suggested the problem and under whose direction the work was carried on.

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THE IMPORTANCE OF TEMPERATURE IN THE USE OF CHEMICALS FOR HASTENING THE SPROUTING OF DORMANT POTATO TUBERS

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INTRODUCTION

In a previous paper² it was shown that early germination of dormant tubers of potato (*Solanum tuberosum* L.) could be obtained by dipping the cut tubers into a dilute solution of ethylene chlorhydrin, and storing the dipped potatoes in a closed container for 24 hours.

Subsequent experiments have shown that the temperature which prevails during the 24-hour storage period after dipping is an important factor. When the temperature was as high as 35° C. (95° F.) severe injury or death of the seed-pieces resulted, and when the temperature was as low as 15° C. (59° F.) the effectiveness of the chemical treatment was considerably lessened. Within the range from 20° C. (68° F.) to 32° C. (90° F.), however, favorable responses were obtained.

Another method of hastening the sprouting of dormant tubers reported upon in the previous paper² consisted in soaking the cut tubers in a dilute (1-2 percent) solution of sodium thiocyanate (NaSCN). It has been found that the temperature of the solution used for soaking the tubers was of less importance with this method. Good results were obtained at temperatures from 15° to 30° C.; some unfavorable influence was observed at 35° but serious rotting of tubers did not result even at this temperature.

The object of the present paper is to show the responses given by dormant potato tubers to chemical treatments at different temperatures.

VARIETY AND SOURCE OF TUBERS

Tubers of the variety Bliss Triumph were used in these experiments. Freshly harvested tubers, dug early in January, 1928, from the fall crop planted in October, 1927, were donated by the Everglades Experiment Station of the University of Florida and were shipped by express to Yonkers.

¹ Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

² Denny, F. E. Second report on the use of chemicals for hastening the sprouting of dormant potato tubers. Amer. Jour. Bot. 13: 386-396. 1926. See also Contrib. Boyce Thompson Inst. 1: 169-179. 1926.

The potatoes were harvested from green vines and, although of fair size (75 to 150 grams), they were immature and therefore very dormant. The degree of dormancy is indicated by the fact that untreated tubers planted January 10 to January 30 showed only a few sprouts on March 20 (text figs. 3, 7, 8).

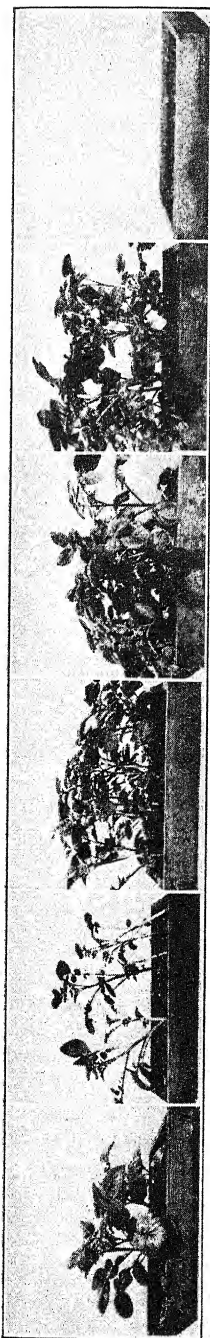
METHOD OF TREATMENT WITH ETHYLENE CHLORHYDRIN

The tubers were first cut into pieces ready for planting. The concentrations of the solutions of ethylene chlorhydrin ($\text{ClCH}_2\text{CH}_2\text{OH}$) used for dipping the cut tubers were varied in order to note the effect of different concentrations at different temperatures. The dipping solutions were prepared by adding 60, 30, 15, and 7.5 cc. of 40-percent ethylene chlorhydrin, respectively, to 940, 970, 985, and 992.5 cc. of water. Fifteen seed-pieces were used in each lot and these were placed in glass Mason jars with a large opening ("EZ-Seal" type). The chlorhydrin solution was then poured in until the jar was full and then poured off at once; the jar was inverted and (after placing the hand over the opening) was shaken to remove the excess liquid; the rubber gasket was applied, and the jar was sealed. The jars were then placed in a constant-temperature oven or water bath and let stand for 24 hours. The treated tubers were planted in shallow flats in soil (15 seed-pieces in each flat), were stored in the dark until sprouting began, and were then placed in a greenhouse maintained at a temperature of 18° C. (65° F.).

RESULTS WITH ETHYLENE CHLORHYDRIN TREATMENTS

In the first experiment the treatments were carried out at temperatures of 10°, 15°, 20°, 25°, 30°, and 35° C. The treatments were applied January 19-20, sprouts appeared above ground February 19-27, and photographs were taken March 20. These results are shown in text figures 1, 2, and 3, and table 1. The photographs show the size attained by the plants in the different lots, but do not show clearly the percentage germination. Information on this point can be obtained from table 1. The lots treated with a dipping solution consisting of 30 cc. of 40-percent ethylene chlorhydrin per liter are shown in text figure 1; those with a dipping solution of 15 cc. per liter are shown in text figure 2; the check lots dipped in water and stored in the same sort of containers for the same length of time and under the same conditions are shown in text figure 3. Other treatments were carried out with dipping solutions consisting of 60 cc. and 7.5 cc. per liter; part of these results are illustrated in text figure 4 and further information regarding them can be found in table 1.

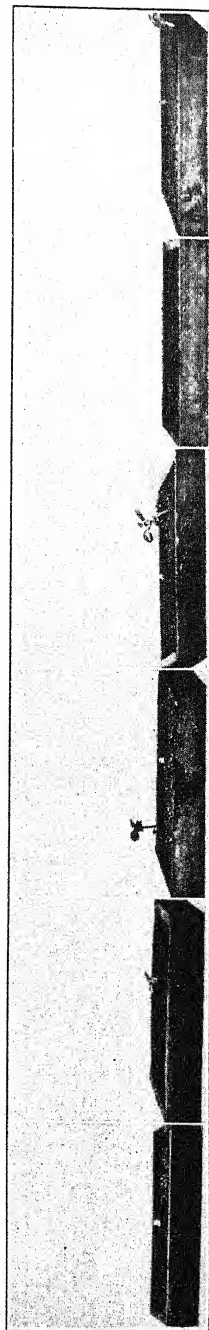
It is important to note the break in the series which occurs between 30° and 35° C. (text fig. 1). A favorable response was obtained at 30° but at 35° the seed-pieces all rotted. At temperatures below 20° C. the treatments using 30 cc. per liter were less successful; however, when the concen-



TEXT FIG. 1. Effect of the temperature during the 24-hour period of storage of potatoes which had previously been dipped into a solution of ethylene chlorhydrin containing 30 cc. per liter. Note the break in the series between 30° C. and 35° C. Compare with the check lots at the corresponding temperatures in text figure 3.



TEXT FIG. 2. Same treatment as in text figure 1 except that the dipping solution contained 15 cc. per liter.



TEXT FIG. 3. Check lots, dipped into water instead of the solution of ethylene chlorhydrin but in all other respects treated in the same way as those shown in text figures 1 and 2. Bliss Triumph variety.

tration of the dipping solution was increased to 60 cc. per liter the germination at 10° C. was improved as is shown by text figure 4 and table 1. The effect of reducing the concentration of the dipping solution to 15 cc. per liter is shown in text figure 2 and table 1. While this series shows much better germinations than the check lots at the same temperatures, the results were not as favorable as those obtained with 30 cc. per liter. Reducing the concentration did not avoid injury at 35° C., and the germinations at the lower temperatures were poor.

TABLE 1. *Influence of Temperature in the Ethylene Chlorhydrin Method of Treating Dormant Potato Tubers*

Temperatures 10° C. to 35° C.					Temperatures 30° C. to 35° C.				
Temp.	Dipping Solution, cc. per liter	Germination Record			Temp.	Dipping Solution, cc. per liter	Germination Record		
		No. Germ.	No. Dormant	No. Rotten			No. Germ.	No. Dormant	No. Rotten
10° C...	60	10	4	1	30° C.	30	15	0	0
" ..	30	5	10	0	" "	15	10	5	0
" ..	15	2	13	0	" Water	4	11	11	0
" ..	Water	0	15	0	31° C.	30	12	2	1
15° C...	30*	7	7	1	" "	15	11	4	0
" ..	15	7	6	2	" Water	1	14	14	0
" ..	Water	1	14	0	32° C.	30	12	1	2
20° C...	60	12	2	1	" "	15	12	2	1
" ..	30	13	2	0	" Water	2	13	13	0
" ..	15	6	8	1	33° C.	30	9	0	6
" ..	Water	1	14	0	" "	15	11	4	0
25° C...	30	12	2	1	" Water	2	13	13	0
" ..	15	9	6	0	34° C.	30	4	0	11
" ..	7.5	10	5	0	" "	15	10	3	2
" ..	Water	1	14	0	" Water	1	14	14	0
30° C...	30	11	3	1	35° C.	30	0	0	15
" ..	15	7	4	4	" "	15	4	2	9
" ..	7.5	7	8	0	" Water	2	12	12	1
" ..	Water	1	12	2					
35° C...	30	0	0	15					
" ..	15	5	1	9					
" ..	7.5	6	4	5					
" ..	Water	0	8	7					

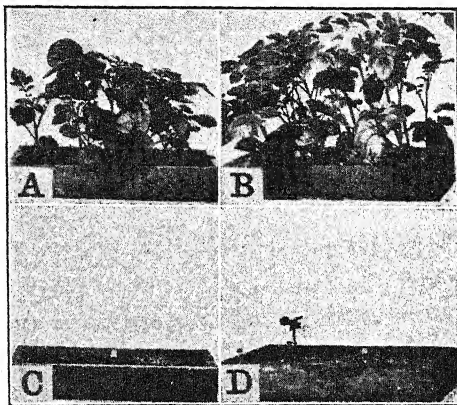
Cut tubers were dipped into a dilute solution of ethylene chlorhydrin and stored 24 hours in a closed container. There were 15 seed-pieces in each lot.

* The 60 cc. per liter lot at 15° C. was destroyed by accident.

Since it was found in the first experiment that the results at 30° contrasted very sharply with those at 35°, a second experiment was carried out in which this range of temperature was investigated at one-degree intervals. This result is shown in text figures 5, 6, and 7, and table 1.

It is seen that with a dipping solution of 30 cc. per liter, the critical temperature is about 32° C. (90° F.), the results being favorable at 30°, 31°, and 32°, and unfavorable at 33°, 34°, and 35°. Reducing the concen-

tration of the dipping solution to 15 cc. per liter (text fig. 6) permitted fair results at temperatures of 33° and 34°, but there was little indication that any considerable gain was obtainable by making treatments with concentrations below 30 cc. per liter.



TEXT FIG. 4. Lots *A* and *B* treated with ethylene chlorhydrin, dipping solution 60 cc. per liter; Lot *A* at 10° C. (40° F.) and lot *B* at 20° C. (68° F.). Lots *C* and *D* checks, dipped in water instead of ethylene chlorhydrin, but in other respects treated the same as lots *A* and *B*; Lot *C* at 10° C. and lot *D* at 20° C.

These experiments with the ethylene chlorhydrin show (with the Bliss Triumph variety, at least) that attention must be given to the temperature which prevails at the time of treatment. The temperature of the place of storage for the treated tubers during the 24-hour period after dipping must be below about 32° C. (90° F.) in order to avoid injury to the potatoes, and should be higher than about 15° C. (59° F.) in order to get maximum percentage germination. The disadvantage of low storage temperature can be partly compensated for by increasing the concentration of the dipping solution to 60 cc. per liter, but injury at temperatures above 32° C. cannot be successfully avoided by decreasing the concentration of the dipping solution.

RESULTS WITH SODIUM THIOCYANATE TREATMENTS

Battery jars of 3.5 liter capacity were nearly filled with the sodium thiocyanate solutions and the temperature of the liquid was properly adjusted before the experimental tubers (previously cut into pieces ready for planting) were added. The temperature was maintained at the desired degree for one hour by constant attention, and the solution was frequently stirred. The treated tubers were then removed and without being rinsed were planted in the soil in flats. After sprouts appeared above ground, the flats were transferred to a greenhouse maintained at about 24° C.



TEXT FIG. 5. Comparison of temperatures within the range 30° C. to 35° C. Ethylene chlorhydrin dip method used, dipping solution 30 cc. per liter; dipped tubers were stored in closed containers 24 hours at the temperatures given above. Note unfavorable results at temperatures above 32° C. (90° F.). Compare with the check lots in text figure 7.

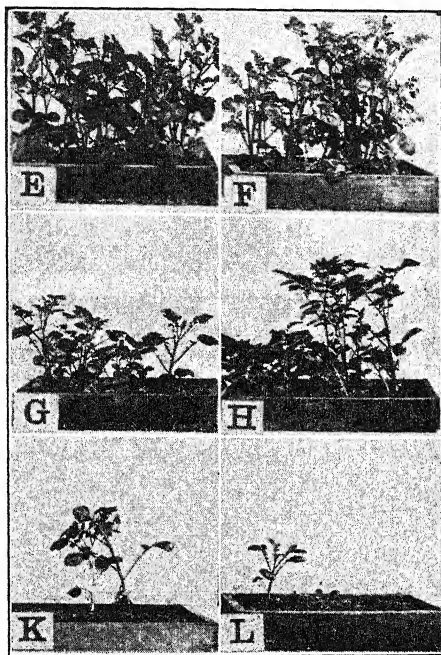


TEXT FIG. 6. Same treatment as in text figure 5, except that the dipping solution contained 15 cc. per liter.



TEXT FIG. 7. Check lots, dipped into water instead of chlorhydrin solution but in all other respects treated in the same way as those shown in text figures 5 and 6. Bliss Triumph variety.

(75° F.). The treatments were made January 27 and the photographs were taken March 20. The results are shown in text figure 8 and in table 2. Only the results at 15° C. and at 30° C. are shown in the figure, but the results at 22° C. were similar in all respects to those at 15° C. and 30° C. In the case of the lots treated at 35° C., it was noted that the tubers soaked in 0.5-percent sodium thiocyanate germinated first, those treated with 1-percent second, and those with 2-percent next. Thus while no serious difficulty with rot was obtained at 35° C. there were indications of some toxicity at this temperature with the stronger concentrations of chemical.



TEXT FIG. 8. Results of treatments with sodium thiocyanate at different temperatures. Lots *E* and *F* soaked 1 hour in two-percent NaSCN, lot *E* at 15° C. and lot *F* at 30° C.; lots *G* and *H* soaked in 0.5 percent NaSCN, lot *G* at 15° C. and lot *H* at 30° C.; lots *K* and *L*, check lots soaked one hour in water, lot *K* at 15° C. and lot *L* at 30° C.

Because of lack of a sufficient quantity of experimental tubers the effect of temperatures below 15° and between 30° and 35° could not be tested in connection with the sodium thiocyanate treatments. The experiments, however, indicated little danger of encountering unfavorable temperatures for these treatments under ordinary conditions.

Attention is specially directed to the favorable germination percentages obtained with the sodium thiocyanate treatments as shown in table 2. It is believed that the layer of sodium thiocyanate which remains on the

tuber after soaking, when rinsing is omitted, acts as a disinfectant and retards the growth of organisms that produce rotting of the tuber. It is proposed to test this point by further experiments.

TABLE 2. *Influence of Temperature in the Sodium Thiocyanate Method of Treating Dormant Potato Tubers*

Temperature	Soaking Solution, g. per liter	Germination Record		
		No. Germinated	No. Dormant	No. Rotten
15° C.....	20	15	0	0
"	10	15	0	0
"	5	14	1	0
"	Water	2	12	1
22° C.....	20	15	0	0
"	10	15	0	0
"	5	15	0	0
"	Water	2	12	1
30° C.....	20	15	0	0
"	10	15	0	0
"	5	15	0	0
"	Water	3	10	2
35° C.....	20	8 *	1	1
"	10	8	1	1
"	5	10	0	0
"	Water	3	7	0

Cut tubers were soaked one hour in a dilute solution of sodium thiocyanate and planted at once without rinsing. There were 15 seed-pieces in each lot.

* Because of insufficient supply of experimental tubers only 10 pieces in each lot were available for the treatments at 35° C.

DISCUSSION

These experiments were carried out with Bliss Triumph tubers in the early stages of the rest period. Other varieties, and this variety in the later stages of the rest period, may be found to respond somewhat differently.

If the injuries to the tuber in the chlorhydrin treatment at temperatures above 32° C. (90° F.) are due either to lack of oxygen or to the accumulation within the closed space of carbon dioxide formed by the high rate of respiration induced under such conditions, it may be found possible to obviate the difficulty by storing the dipped tubers under canvas instead of in closed containers. This would permit better aeration during the period of storage. In such a case it may be necessary to increase the concentration of chemical.

In this experiment sodium thiocyanate gave better results than ethylene chlorhydrin. In the hundreds of experiments that have been carried out with these two chemicals the results are sometimes better with one and sometimes with the other. In order to determine the factors which cause this difference in behavior, it is planned to carry out a series of experiments in the summer of 1928.

Although emphasis in this paper has been placed upon the conditions

under which unfavorable results can be obtained, it is evident from an examination of the figures and the tables that the previous reports regarding the capacity of these two chemicals to hasten the sprouting of dormant tubers have been confirmed. The temperatures and concentrations at which treatments were carried out were varied over a wide range in order to find the conditions that were unfavorable, yet almost all of the treated lots were better than the checks. The 16 check lots showed in all an average germination of 11 percent. The 41 treated lots, including all concentrations and all temperatures, gave 66 percent germination; and if only the treatments under favorable conditions are considered, *i.e.* ethylene chlorhydrin with a dipping solution of 30 cc. per liter at temperatures of 20° C. to 32° C., and sodium thiocyanate using a one-percent solution at temperatures of 15° C. to 30° C., the germination percentages are: for ethylene chlorhydrin, 83 percent, and for sodium thiocyanate, 100 percent.

SUMMARY

1. This is a continuation of experimental work on the use of ethylene chlorhydrin and sodium thiocyanate for shortening the rest period of potatoes. The object of this experiment was to determine with greater accuracy the influence of the temperature during the process of treating the tubers.

2. When the germination of dormant potato tubers was hastened by dipping the cut tubers into a dilute solution of ethylene chlorhydrin ($\text{CICH}_2\text{CH}_2\text{OH}$) and storing the dipped tubers in closed containers for 24 hours, the temperature which prevailed during the 24-hour storage period was found to be an important factor.

3. Using a dipping solution obtained by adding 30 cc. of 40-percent ethylene chlorhydrin to 970 cc. of water favorable results were obtained at 20°, 25°, and 30°, but at 35° C. (95° F.) the seed-pieces were killed by the treatment and rotted subsequently. The range from 30° C. to 35° C. was investigated at one-degree intervals. Good results were obtained at 30°, 31°, and 32° C. (90° F.); but at 33°, 34°, and 35° C., low percentage germinations due to rotting of tubers resulted.

4. At temperatures below 20° (68° F.) a dipping solution of 30 cc. per liter was only partly effective and many seed-pieces remained dormant; better germination percentage was obtained when the concentration was increased to 60 cc. per liter.

5. Experiments were also carried out on the effect of temperature in the sodium thiocyanate method of treating dormant tubers. This consists in soaking the cut tubers for one hour in a dilute solution of sodium thiocyanate (NaSCN). It was found that the temperature of the solution used for soaking the tubers was of less importance with this method. Good results were obtained at 15° C. (59° F.), 22° C., and 30° C. (86° F.) and with all three concentrations of chemical used in the experiment: two-

percent, one-percent, and one-half-percent sodium thiocyanate. At 35° C. (95° F.) some evidence of toxicity was observed at the highest concentration, but no serious difficulty with rotting of tubers resulted.

6. These experiments were carried out with tubers of the Bliss Triumph variety. The experimental tubers were harvested from green vines and were in a deeply dormant condition.

7. Previous experiments showing that treatments with these chemicals hastened the sprouting of dormant potato tubers were confirmed. Untreated tubers showed 11-percent germination 7-8 weeks after planting. The average of all chemical treatments at all concentrations and all temperatures was 66 percent, and the average for the chemical treatments under favorable conditions was 83 percent for the ethylene chlorhydrin treatments, and 100 percent for the sodium thiocyanate treatments.

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EFFECT OF THE AGE OF POLLEN UPON THE SEX OF HEMP

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In 1871 Dr. Theophilus Ciesielski undertook experiments at the University of Vratislav in the attempt to discover the factors responsible for the determination of sex in hemp (*Cannabis sativa*). The effects of close and distant planting were tested with negative results. In 1872 the date of sowing, with reference to the moon's phases, was shown to have no effect. From 1873 onward the experiments were continued at the University of Lwow (Lemberg). The effects of full light and shade, of well fertilized and of sterile soil, of abundance of moisture and of drought were investigated, but though the percentage of male plants varied between 40 and 50 percent there was no evidence of any influence of these conditions upon the sex ratio. Seeds from the upper, middle, and lower parts of the inflorescence gave the same sex ratios. In 1876 all male plants were removed from two parts of his garden. In one part the female plants were artificially pollinated each day at sunrise and in the other part about sunset with pollen collected in the morning. The two lots of seeds were harvested separately and planted in 1877. Those from the plants pollinated early gave 85.5 percent male plants while the plants produced by the seeds that were pollinated at sundown gave 92 percent female plants. The experiment was repeated in 1877, this time six female plants being taken indoors before blossoming, to avoid chance of wind-borne pollen affecting the results. The flowers were pollinated with a brush. Of the 112 plants obtained from the seeds produced by pollination with pollen removed from anthers that had just begun to open, 106 were male; while of the 89 coming from seeds produced on plants pollinated with 12-hour-old pollen, all were female. These experiments are reported by Ciesielski to have been repeated several years, always with the same results. He experimented also with animals, but his results do not interest us here.

Lilienfeld, in 1921, reported the results of a series of experiments made by him with the express purpose of testing the accuracy of Ciesielski's results. He pollinated only a few plants but used special precautions to

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avoid contamination. Offspring from plants pollinated with fresh pollen did not differ in sex ratio, to any significant degree, from offspring from plants pollinated with twelve-hour, thirty-hour, and thirty-six-hour pollen. The figures were, respectively: percentage of male plants, 37.8 percent, 38.5 percent, 45.1 percent, and 40.2 percent. The fresh pollen gave minimum and maximum percentages of males from different mother plants of 32.25 percent and 48.91 percent, respectively, the corresponding figures for twelve-hour pollen being 37.4 percent and 42.86 percent.

My experiments were begun several years ago but for the first two or three years no results of worth could be obtained because of the impossibility of obtaining seed of good germinating value. With seed yielding less than 10-percent germination the results were questioned. At last in the fall of 1924 some excellent seed was obtained and experiments were begun in the greenhouse. The difficulty of obtaining sufficient illumination made the plants remain small and as a result comparatively few seeds were obtained. These were germinated and the resulting plants were set out in the Botanical Garden early in the summer of 1925. Of these few plants the sex ratios were about the same for those from seed resulting from pollination with fresh pollen as for those coming from 12-hour-old pollen. Large numbers of seedlings were grown from the main seed stock directly in the beds in the Garden and these were used mainly in the pollination experiments. Owing to my absence at the beginning of the blossoming period the earlier developed female flowers were open-pollinated. On the plants chosen for the experiments, certain branches were selected and all flowers that showed signs of pollination were removed. The branches were then bagged with large paper sacks. After one or two weeks the sacks were removed. At this time the flowers that had already been pollinated when the bagging was done, but which at that time did not show any signs of it, now showed by the increased size of the ovary that they had actually been pollinated. These flowers, too, were removed. The experiment was then carried on in the following manner. The pollen which was to be used was contained in a paper sack. This was tied around the branch, enclosing it, and was then shaken and beaten until every receptive stigma had a chance to receive pollen. The bag was allowed to remain until such time as all male plants had been destroyed. The foregoing method was always employed when old pollen was used and sometimes with fresh pollen. In some cases anthers that were just on the point of opening or that had opened just a trifle were picked off with forceps and the contents shaken out over the clusters of female flowers, these being bagged afterwards.

In 1925 the pollinations were accomplished mostly between September 3d and September 11th, the bagging of the inflorescences having taken place August 29th. On each plant upon which pollinations took place the remainder of the branches were left uncovered to permit of open pollination, thus allowing the open-pollinated seed from the same plant to serve as a control for the comparison of sex ratios of the progeny.

All male plants were destroyed September 11th and 12th and all bags were removed from the pollinated branches on the 14th, so as to permit of normal development of the seeds. As the nearest hemp plants from which pollen might possibly come were in a patch about half a mile away, in which nearly all the male plants were dead, the chance of accidental pollination was considered negligible. The seeds from the specially pollinated shoots and from open-pollinated shoots on the same plant were harvested October 30th and preserved in paper bags until planting time in 1926. Planting was done May 12th and 13th, 1926, in the Botanical Garden of the college. On August 6th the records were made as to the sex of the plants produced, while on the 25th similar records were taken on three large plats of plants grown from seed from open-pollinated plants of the previous year, these plants not being ones upon which pollination experiments were made, but of the same original lot of seed.

The results of the individual pollinations are shown in table 1. They may be summarized for the 1925 pollinations as follows: Pollinations on five different plants early in the morning with fresh pollen produced seeds from which 210 plants grew, of which 43.8 percent were male. Open-pollinated flowers from the same plants gave seeds that produced 310 plants, of which 49.7 percent were male. Two similar pollinations late in the afternoon, but with fresh pollen, gave 107 plants of which 44.9 percent were male, while the checks from the same plants gave 43 plants with 46.5 percent male. Using "old" pollen, varying from 8 or 9 up to 64 hours old, there resulted 549 plants of which 43.4 percent were male. These included pollination upon ten different plants. The open-pollinated flowers upon the same plants gave rise to 517 plants of which 48.7 percent were male. Another group of three open-pollinated plants gave seeds which produced 1307 plants of which 44.5 percent were male. There was, however, considerable variability in the sex ratio of the offspring of the three plants, the proportion of males being, respectively, 42.3 percent, 42.2 percent, and 49.5 percent. Similarly in one experiment with pollination with 14- to 16-hour-old pollen the proportion of males was 39.1 percent while in another experiment with pollen of similar age the males made up 54.8 percent of the offspring. The checks for these two experiments from open-pollinated branches of the same plants showed, respectively, 51.2 percent and 55.6 percent males. Attention may be called here to the rather strange fact that all of the artificially pollinated shoots gave, when totaled without regard to the age of the pollen, 43.65 percent male plants; while the open-pollinated checks from the same plants gave 49.0 percent male plants.

It is clear, from the foregoing, that for the 1925 pollinations there is no distinguishable effect of age of pollen upon the sex of the offspring.

In 1926 pollinations were accomplished in about the same manner as in 1925. The dates of pollination were August 26th and 27th. The male

TABLE 1. *Number of Male and Female Plants in 1926 as a Result of 1925 Pollinations. Each Line Shows at the Left of the Table the Results of a Single Experiment and at the Right of the Table the Ratios from Open-pollinated Shoots of the Same Mother Plant*

Expt. No.	Male	Female	Control No.	Male	Female
Pollination in morning with fresh pollen					
26-3	40	42	26-4	30	30
26-10	2	0	26-11	65	66
26-5	0	0	26-2	21	20
26-29	43	73	26-28	20	21
26-21	7	3	26-22	18	19
Total	92 (43.8%)	118 (56.2%)		154 (49.7%)	156 (50.3%)
Pollination in late afternoon with fresh pollen					
26-8	22	22	26-9	1	1
26-23	26	37	26-24	19	22
Total	48 (44.9%)	59 (55.1%)		20 (46.5%)	23 (53.5%)
Pollination in late afternoon with 8-9-hour pollen					
26-12	24	39	26-15	22	20
26-26	1	0	26-27	37	37
Total	25 (39.1%)	39 (60.9%)		59 (50.9%)	57 (49.1%)
Pollination in morning with 14-16-hour pollen					
26-1	18	28	26-2	21	20
26-6	23	19	26-7	25	20
Total	41 (46.6%)	47 (53.4%)		46 (53.5%)	40 (46.5%)
Pollination in morning with 24-hour pollen					
26-34	74 (38.1%)	120 (61.9%)	26-35	30 (54.5%)	25 (45.5%)
Pollination in late afternoon with 24-hour pollen					
26-13	3	6	26-15	22 (52.4%)	20 (47.6%)
26-14	2	1			
Total	5 (41.7%)	7 (58.3%)			
Pollination in morning with 42-hour pollen					
26-32	82 (50%)	82 (50%)	26-33	73 (42.2%)	100 (57.8%)
Pollination in morning with 64-hour pollen					
26-19	9	13	26-20	2	2
26-31	2	3	26-28	20	21
Total	11 (40.7%)	16 (59.3%)		22 (48.9%)	23 (51.1%)

TABLE I (continued)
Totals of the foregoing

	378 (43.65%)	488 (56.35%)		426 (49%)	444 (51%)
Totals for "fresh" pollen					
	140 (44.2%)	177 (55.8%)		174 (49.3%)	179 (50.7%)
Totals for "old" pollen					
	238 (43.4%)	311 (56.6%)		252 (48.7%)	265 (51.3%)
Open-pollinated plants					
26-37	195	266			
26-38	183	251			
26-39	204	208			
Total	582 (44.5%)	725 (55.5%)			

plants were all removed September 8th and the bags removed from the pollinated shoots two days later. The seeds were harvested October 15th and preserved as in the previous year. Owing to unfavorable conditions the seeds could not be planted until June 16th, 1927, but the plants reached full flowering by August 29th, with but very few exceptions. At that date the records were taken (table 2).

TABLE 2. Number of Male and Female Plants in 1927 as a Result of 1926 Pollinations. Each Line Shows at the Left of the Table the Results of a Single Experiment and at the Right of the Table the Ratios from Open-pollinated Shoots of the Same Mother Plants

Expt. No.	Male	Female	Control No.	Male	Female
Pollination with fresh pollen					
13-26-1-1 C	15	21	13-26-1-2 O	5	12
31-26-2-3 C	2	7	31-26-2-4 O	11	11
39-26-3-5 C	18	14	39-26-3-6 O	14	20
38-28-1-17 C	61	63	38-28-1-18 O	90	60
29-28-2-19 C	23	28	29-28-2-20 O	1	2
Total	119 (47.2%)	133 (52.8%)		121 (53.5%)	105 (46.5%)
Pollination with 10-12-hour pollen					
13-26-4-7 C	0	1	13-26-4-8 O	25	27
31-26-5-9 C	12	8	31-26-5-10 O	0	1
21-26-6-11 C	45	57	21-26-6-12 O	30	39
39-26-7-13 C	138	82	39-26-7-14 O	60	51
27-28-3-21 C	20	22	27-28-3-22 O	2	1
Total	215 (55.8%)	170 (44.2%)		117 (49.6%)	119 (50.4%)
Pollination with 17-27-hour pollen					
39-27-1-15 C	32 (47.1%)	36 (52.9%)	39-27-1-16 O	46 (50%)	46 (50%)

The results for this second year may be summarized as follows: 252 plants resulting from pollination with fresh pollen consisted of 47.2 percent males and 52.8 percent females, while the 226 checks from open-pollinated branches of the same parent plants had the ratio 53.5 percent male and 46.5 percent female. 385 plants resulting from pollination with pollen 10 to 12 hours old had the ratio 55.8 percent males and 44.2 percent females, the check ratio being, respectively, 49.6 percent to 50.4 percent. 68 plants produced as a result of using pollen 17 to 27 hours old had 47.1 percent males and 52.9 percent females, the 92 checks being equally divided between males and females. Here there is a small difference in the ratios obtained, amounting to a deviation from the control figures of 6.3 percent less males for the progeny of flowers pollinated with fresh pollen and of 5 percent more males where old pollen was used. This deviation is in the direction contrary to that reported by Ciesielski. The significance of these small deviations seems small when the results of individual pollinations are compared. One pollination with fresh pollen gave 32 plants, with 56.3 percent males; another gave 124 plants of which 49.2 percent were males; and a third gave 36 plants, with 41.7 percent males, a difference between the extremes of 14.6 percent. In the series of plants resulting from the use of pollen 10 or more hours old we find one pollination whose results gave 102 plants with a ratio of 44.1 percent male. Another similar pollination resulted in 220 plants of which 62.7 percent were male. Here the difference is 18.6 percent. It is clear, then, that the deviations from the average of the control actually observed are not great enough to be considered as of much significance.

My results confirm those of Lilienfeld and are not in accord with the findings of Ciesielski. I am unable to reconcile the divergent results. Hemp has been shown by Schaffner and by Pritchard to be subject to sex reversal under conditions of modification of the environment and possibly the lability thus exhibited is also present when the environment is modified in other ways. At least, however, one may say that for the sorts of hemp studied by Lilienfeld and by the writer, there is no evidence that the age of the pollen has any effect upon the sex of the offspring.

SUMMARY

Female flowers of hemp were artificially pollinated in 1925 and 1926 with pollen of various ages. The seeds thus produced were planted the following year. The sex ratio of the plants grown from these seeds was not significantly different, when the pollen used was taken from anthers just beginning to open, from what it was when the pollen had been collected hours before. This is in direct contradiction to the results of Ciesielski's experiments in which fresh pollen produced seeds that gave rise to male plants and old pollen led to the production of female plants.

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A STUDY OF THE EFFECT OF HOT AND COLD WEATHER ON THE CATALASE OF THE PLANT AND ANIMAL IN RELATION TO THEIR RESPIRATORY METABOLISM

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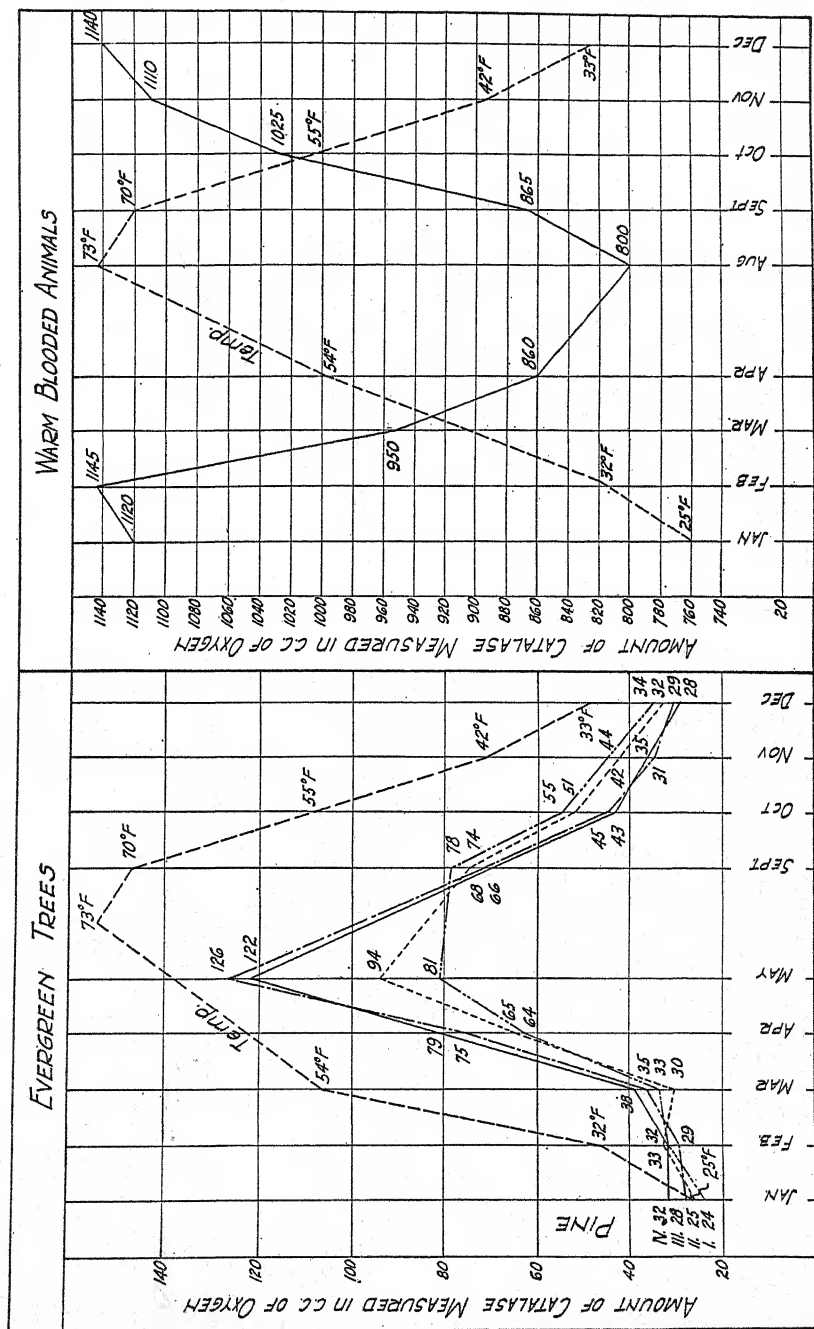
It is known that cold weather decreases the respiratory metabolism in plants and cold-blooded animals and increases it in warm-blooded animals. The present investigation is an attempt to find an explanation for this effect of temperature on metabolism.

Respiratory metabolism or oxidation is one process that goes on in all living things, animals as well as plants. There is one enzyme, catalase, that is well-nigh universally present in all living things. This enzyme possesses the property of liberating oxygen from hydrogen peroxid, which suggests that it may be connected with oxidation or the respiratory metabolism. Therefore in looking for an explanation for the effect of temperature on the respiratory metabolism one naturally turns to catalase.

The relation between catalase content and respiration in plants as well as in animals has been the subject of a great number of investigations. Appleman (1) found an increase in catalase corresponding with the increase in respiration in the greening and sprouting of potatoes. Crocker and Harrington (2) found a similar relation between catalase and respiration in the germination of seeds. It is known that fertilization greatly increases oxidation or the respiratory metabolism of the ovum. Winternitz (3) found that the unfertilized hen's egg showed very little catalactic activity even after prolonged incubation whereas the incubated fertilized egg rapidly acquired the power of decomposing hydrogen peroxid. It is also known that the respiratory metabolism of the newly born is low and that it increases in youth and decreases in old age. Battelli and Stern (4) found that the catalase content of most of the tissues, particularly of the liver, of newly born pigs was lower than the corresponding tissues of the mother, but that the catalase rapidly increased until at the end of the seventh or eighth day it was as high as that of the adult.

The plant used in this investigation was *Pinus Strobus*, large trees being studied. The warm-blooded animals used were ordinary white rabbits.

The needles from four pine trees were collected weekly throughout the year, brought to the laboratory, and ground twice through a small hashing machine. Precautions were taken to see that only needles of the previous year were used. One gram of this material was added to 25 cc. of neutral



TEXT FIG. 1. Curves showing that warm weather increases the catalase of evergreen trees and decreases the catalase of warm-blooded animals, and that cold weather decreases the catalase of evergreen trees and increases the catalase of warm-blooded animals, thus paralleling the effect of temperature on metabolism.

hydrogen peroxid, and the amount of oxygen liberated in ten minutes was taken as a measure of the catalase content of the needles. The results of the determinations are given in text figure 1 under "Evergreen Trees." It will be seen that the average amount of oxygen liberated by the needles of Pine I during January was 24 cc., that by Pine II, 25 cc., that by Pine III, 28 cc. and that by Pine IV, 32 cc. Similarly there may be seen in the chart the average amount of oxygen liberated by the needles of the different pines for February, March, April, May, September, October, November, and December. There also appears here the curve of the average temperature for the different months.¹ From the preceding it may be seen that the catalase content of the pine needles was lowest during the months of January and February when the weather was coldest, and as it became warmer, passing from winter into spring and summer, the catalase rose and was highest in the summer when the weather was hottest. As the weather became cooler, passing from summer into fall and winter, the catalase decreased, and by December had returned almost to the level found during the preceding January. It has also been shown that low temperatures decrease the sugar metabolism and catalase content of *Spirogyra* (5).

In making the determinations of the catalase content of the blood of rabbits, 1 cc. of the blood taken from the jugular or ear vein, diluted 1 : 3 with physiological saline, was added to 150 cc. of neutral hydrogen peroxid and the amount of oxygen liberated in ten minutes was taken as a measure of the catalase content of the blood. These rabbits were kept in an open court and hence exposed to the same temperature as were the pines. The average results for the determinations for the different months are shown in text figure 1 under "Warm-Blooded Animals." The curve for the average temperatures for the different months is also given. It will be seen that the blood catalase was highest in the months of January and February when the weather was coldest and as the weather grew warmer, passing from winter into spring and summer, the catalase decreased and was lowest in August when the weather was warmest, and as the weather grew colder, passing from summer into fall and winter, the catalase increased and by December had returned almost to the level found during the previous January and February. The question whether these results obtained with rabbits will hold for man might be raised. We see no reason why they would not if man is exposed to the cold. Ordinarily, however, man protects himself against the cold by means of additional clothing and warm quarters, so it is doubtful if cold weather increases metabolism in man very much. It should be mentioned that low temperatures decrease the blood catalase of the turtle (6), a cold-blooded animal, corresponding with its effect on metabolism.

¹ These temperatures were taken from the records of the weather bureau at the University for the year 1925, when this work was done.

SUMMARY

1. The catalase of the needles of pine trees is decreased in the winter and increased in the summer corresponding with the decrease in metabolism in cold weather and the increase in warm weather.

2. The catalase of the blood of rabbits, on the contrary, is increased in the winter and decreased in the summer, corresponding with the rise in metabolism in cold weather and decrease in hot weather in the rabbit.

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DEVELOPMENT OF THE EMBRYO SAC AND YOUNG EMBRYO OF *HICORIA PECAN*¹

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INTRODUCTION

Previous studies on flower and nut development in *Hicoria pecan* Brit. have shown that the post-pollination period is a critical one and justifies a detailed study. It is believed that numerous hitherto unanswered questions of popular interest, relative to various types of abortion and "dropping" of nuts, can be explained when the morphological changes accompanying pollination and fertilization are fully understood. The purpose of the present report is to describe the development of the embryo sac and the young embryo and to trace the course of the pollen tube, recording the time when all important stages occur. This paper is the fourth of a series on the development of the pistillate flower and nut of the pecan (4, 5, 6).

MATERIALS AND METHODS

Materials for microscopic study have been collected during the past three years. The period covered was from about a week prior to receptivity (about April 25th) until August 25th. The interval between collections varied from six hours in the case of hand-pollinated flowers of the Rome and Stuart varieties to 24 hours in the case of Alley and Nelson varieties, and to five days in the case of Frotscher and Beverage varieties.

The killing and fixing solutions used were the same as in previous studies (4, 5). All material was imbedded in paraffin and sectioned ten microns in thickness. Flemming's triple stain and Pianeze's stain were tried but failed to show the differentiation obtained with Heidenhain's iron-alum haematoxylin.

RESULTS

Development of the Embryo Sac

In the pecan, megaspore mother cells are present in the ovules of flowers collected during the week preceding pollination (Pl. XXIII, fig. 4). The mother cells are formed prior to the appearance of the single integument, which does not occur until after the flowers are seen above the unfolding leaves early in April.

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In *Juglans cordiformis*, Karsten (2) found great variability in the number of megaspores. In some cases embryo sacs developed directly from megaspore mother cells; in others a row of three or four megaspores was formed. The two outer megaspores did not function but apparently the two inner ones had an equal chance for development. In some cases two embryo sacs were observed in a single nucellus.

In the pecan four megaspores seem to be the rule. Observations indicate that the four-megaspore stage is reached at the same time that the stigmas become receptive. The megaspores are usually arranged in an axial row though sometimes the two most apical ones have been observed lying side by side. The innermost or chalazal megaspore functions as an embryo sac, while the remaining three rapidly disintegrate, becoming a dark shapeless mass before the first nuclear division occurs in the chalazal megaspore. Mere traces of these remain at the time of the second division of the embryo sac nuclei (Pl. XXIII, figs. B, C, D, E).

The embryo sac enlarges and approximately doubles in size after each successive nuclear division. The expanding sac depends on the surrounding nucellus for nourishment. The chalazal megaspore is pointed at the base and continues so until after fertilization takes place. The upper two-thirds of the sac is oval to round in shape (Pl. XXIII, figs. B, C, D, E, F, G, H).

The first nuclear division takes place near the center of the embryo sac, followed by the migration of the two daughter nuclei, one to each end of the embryo sac. Before the migration of the two nuclei two comparatively large vacuoles are present, but following migration each embryo sac contains only one large vacuole (Pl. XXIII, figs. E, F).

The time elapsing between the four-megaspore stage and the last nuclear division in the embryo sac is about one week. The third division produces an eight-nucleate embryo sac; this division is followed immediately by a fusion of the polar nuclei in the center of the sac (Pl. XXIII, fig. G).

After this fusion the egg nucleus enlarges somewhat and the synergid nuclei remain unchanged, or may even show signs of disintegration. The synergid nuclei are always located toward the apex of the embryo sac. At this stage cell divisions apparently do not occur in the micropylar end of the embryo sac. Consequently the synergid nuclei and the egg nucleus lie in the same cytoplasmic mass, which is continuous with the cytoplasm about the polar nuclei. In one instance in which fertilization had been delayed, two distinct synergids were seen, both slightly to one side of the apex of the embryo sac. If this condition is of regular occurrence it must be of very short duration, and it has not been observed in what seem to be typical cases. The egg nucleus occupies a more central position than the synergid nuclei, and near the fusing polar nuclei. Before fertilization the egg nucleus moves to the micropylar end of the embryo sac and a cell division occurs which delimits the egg as a distinct cell, no separate syner-

gids, however, being formed (Pl. XXIV, fig. B). A similar condition as to organization of the synergids and egg is described by Nawaschin (3) and Karsten (2) in several species of *Juglans*.

Fusion of the polar nuclei is complete about ten days after pollination, a large fusion nucleus or primary endosperm nucleus being formed in the unfertilized embryo sac. This enlarges considerably before it divides. It is the largest of the embryo-sac nuclei and is located in the lower part of the dense cytoplasm that surrounds the egg and synergid nuclei in the apical half of the embryo sac. A large branched strand of cytoplasm attaches the endosperm nucleus to a point near the base of the embryo sac (Pl. XXIII, fig. H). Karsten (2) found that there is no fusion of polar nuclei in *Juglans nigra* or that if it does occur it is very late.

The three antipodal cells are practically cut off from the embryo sac, having been forced into the point at the base, in which position they remain unchanged until after fertilization (Pl. XXIII, figs. G, H).

Course of the Pollen Tube

The course followed by the pollen tube of the pecan differs in certain respects from that described by Nawaschin (3) for *Juglans*, Billings (1) for *Carya olivaeformis* (*Hicoria pecan* Brit.), and Karsten (2) for *Juglans* and *Carya amara*. The course of the pollen tube as described by these workers is best summarized by Billings:

The pollen tube passes down the axial tissue of the style till near the cavity of the ovary, where it turns and passes down the ovary wall close to the margin of the cavity. The tissue through which it passes after leaving the style has nothing by which it could be designated "conducting tissue," but consists of nearly isodiametric cells. When a point is reached a little below the funiculus, the pollen tube curves, passes through the region of deeply stained cells (as though mucilaginous), and when under the ovule turns upward toward the embryo sac.

In the pecan the writer has found that the pollen tubes grow inward from the sides of the stigma toward the micropyle. Numerous pollen grains germinate on the stigmatic surface and send tubes into the tissue. However, the tubes never enter the stylar canal but grow downward in the tissue on each side of the micropyle and so approach the ovule opposite the plane of the middle septum or placenta (Pl. XXV, figs. D, E, F). Thus it is necessary for them to cross the narrow crevices which extend downward nearly to the base of the flower on each side and parallel to the plane of the placenta.

Pollen tubes enter the cavity of the ovary within six to twelve hours after the hand-pollination of the stigmas. They often reach the cavity as early as the four-megaspore stage. The place of entrance into the cavity of the ovary is usually near the micropyle, though they have been found entering at a point nearly opposite the chalaza. However, they do not enter the nucellus until the embryo sac is mature, about a week later,

but continue growing about in the cavity and crevices. They branch very profusely in all directions. Following the maturing of the embryo sac the tubes enter the nucellus at the base of the integument apparently always a little to one side of the fibrovascular bundles, passing through the less resistant tissues and not through the dense tissue of the bundles. Tubes have been observed crossing the space above the ovule apparently to enter the nucellus at the micropylar end before the embryo sac is mature.

Billings (1) describes the integument of *Carya olivaeformis* as tightly inclosing the nucellus at the time of pollination. Varieties used in this investigation have the nucellus only about one-half inclosed by the time the embryo sac becomes mature. The length of the integument varies to some extent with the variety.

Fertilization

The pollen tube enters the micropylar end of the embryo sac about two weeks after pollination. In some cases its entrance is probably even later. Fusion of the polar nuclei always precedes the entrance of the pollen tube into the embryo sac. One of the male nuclei fuses with the endosperm nucleus immediately after the pollen tube enters. Free nuclear division of the endosperm nucleus occurs directly after this triple nuclear fusion. The endosperm is thus formed from a cell containing a triple-fusion nucleus, whose division results in the formation of many endosperm nuclei lying in the peripheral cytoplasm of the embryo sac; no cell division occurs between these nuclei (Pl. XXIV, figs. B, D).

The antipodal cells and synergid nuclei disintegrate immediately after the entrance of the pollen tube into the embryo sac.

After the entrance of the pollen tube, the egg nucleus begins migration toward the micropylar end of the sac where, as already noted, a definite egg is delimited, which becomes attached at the apex of the embryo sac. The second male nucleus has been observed near the egg nucleus five weeks after pollination. The actual fusion of the egg and male nuclei probably occurs, as a rule, about two to three weeks after the pollen tube has entered the embryo sac. A fertilized egg has been observed with the dead end of a pollen tube still present seven weeks after pollination (Pl. XXIV, figs. B, C). Pollen tubes remain in the embryo sac two to three weeks or even longer before they disintegrate.

Development of the Embryo

The fertilized egg does not undergo division earlier than about two months after pollination. Early in July it divides horizontally, forming a large one-celled suspensor. The second or chalazal cell continues division in such a manner as to produce a round globular embryo attached at the micropylar end of the sac by the large suspensor cell. The cotyledons begin developing about one month after the first division (Pl. XXIV,

figs. E, F, G). The later stages in the development of the embryo have been described in previous papers (5, 6). About three months elapse between the first division of the fertilized egg and the maturing of the nut (5, 6). This time varies somewhat, depending on the variety.

SUMMARY AND CONCLUSIONS

In the present paper the normal development of the embryo sac and the embryo of the pecan under Georgia conditions have been described. The dates and related stages will vary somewhat under a different climatic environment. In the course of the work numerous abnormal conditions have been met and studied in some detail; but it is believed that a thorough understanding of the normal behavior is necessary before an attempt is made to explain any abnormalities. Consequently, all statements as to abnormal conditions are being reserved until further studies have been made.

The findings differ somewhat from those of Karsten, which may be explained by the fact that he worked with other species and genera of the family Juglandaceae. They also differ somewhat from those of Billings, whose only available report does not include the methods used nor a detailed account of his results, so that an explanation of the differences cannot well be suggested.

Four megaspores seem to be the invariable number in *Hicoria pecan* Brit., and they appear simultaneously with the receptivity of the stigmas and pollination. A week later the embryo sac becomes mature and contains eight nuclei. The polar nuclei fuse within three days after maturity of the embryo sac.

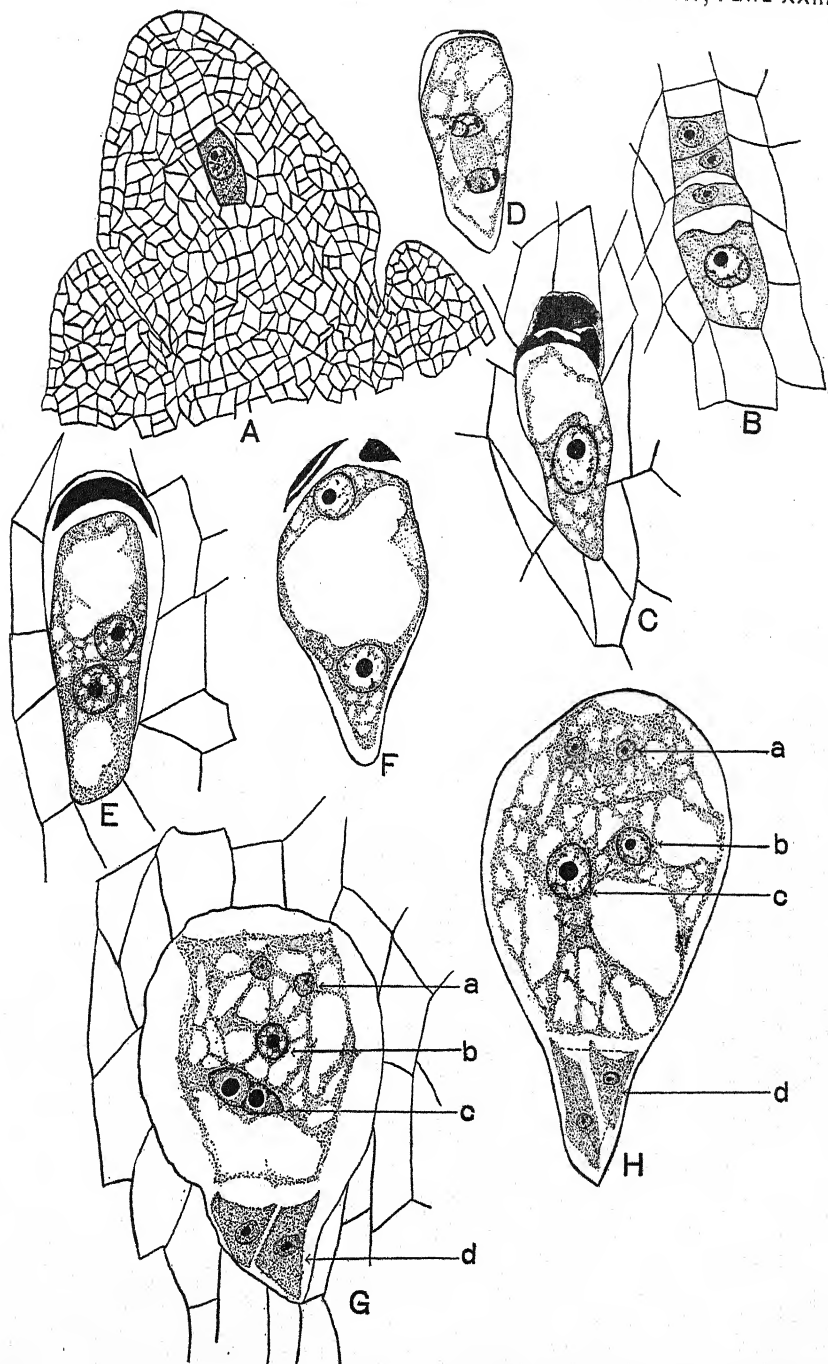
The pollen tube enters the embryo sac about two weeks after pollination. Its entrance is followed immediately by the fusion of one of the male nuclei with the primary endosperm nucleus, which in turn undergoes free nuclear division to form endosperm nuclei.

The egg and second male nucleus fuse during the fifth or sixth week after pollination, and the first division of the fertilized egg occurs about two months after pollination.

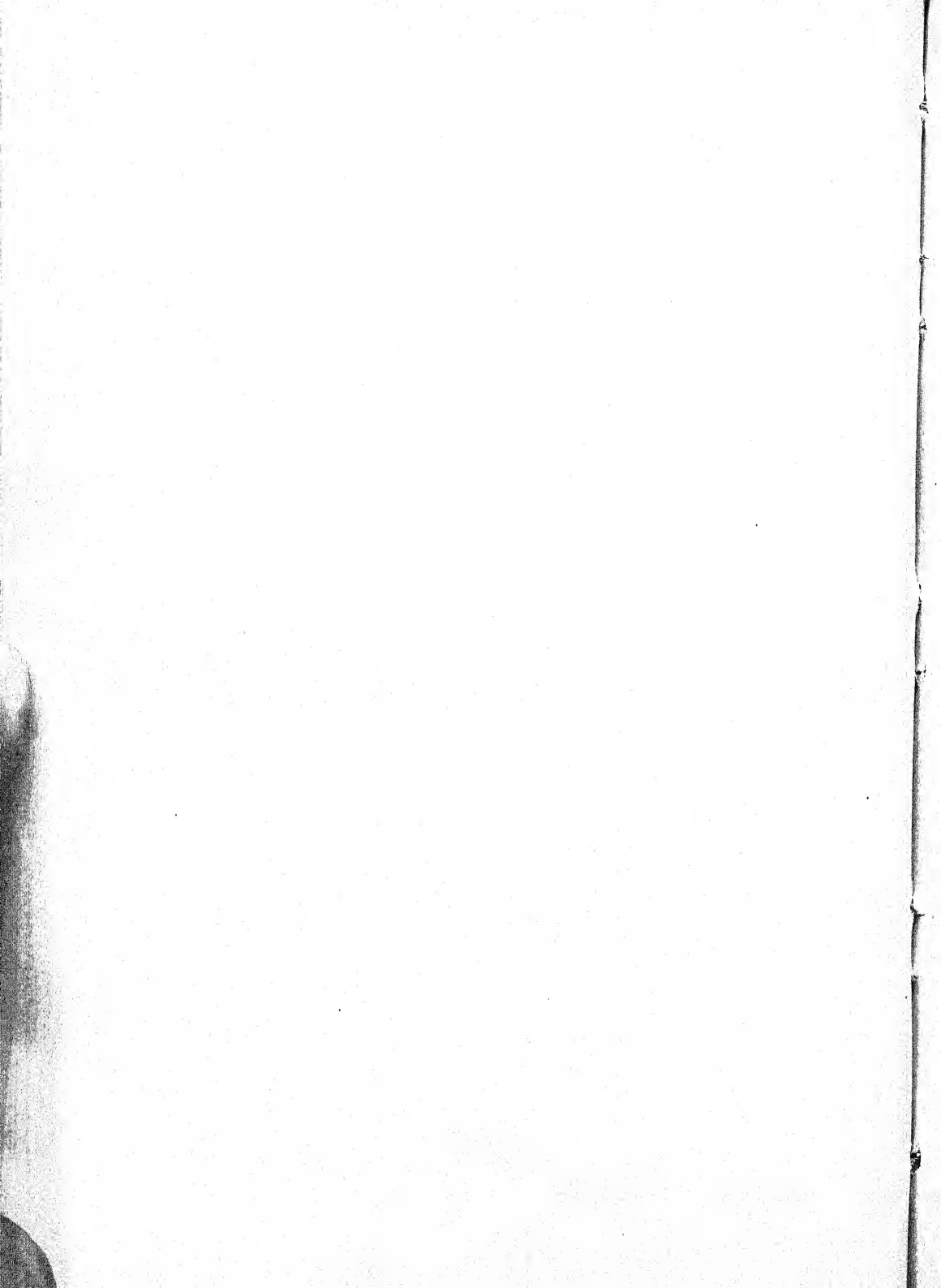
The writer wishes to express her appreciation to Dr. B. B. Higgins for his valuable advice and constructive criticisms during the progress of this work, and to Dr. C. E. Allen for reading the manuscript.

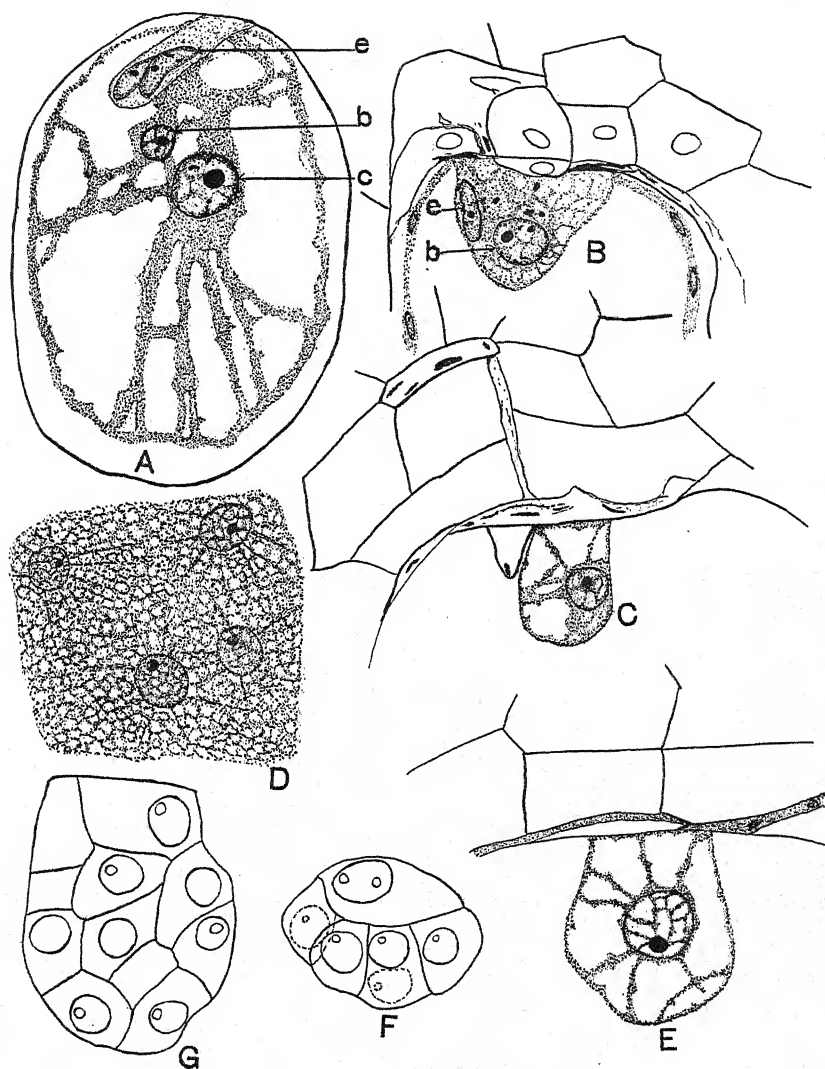
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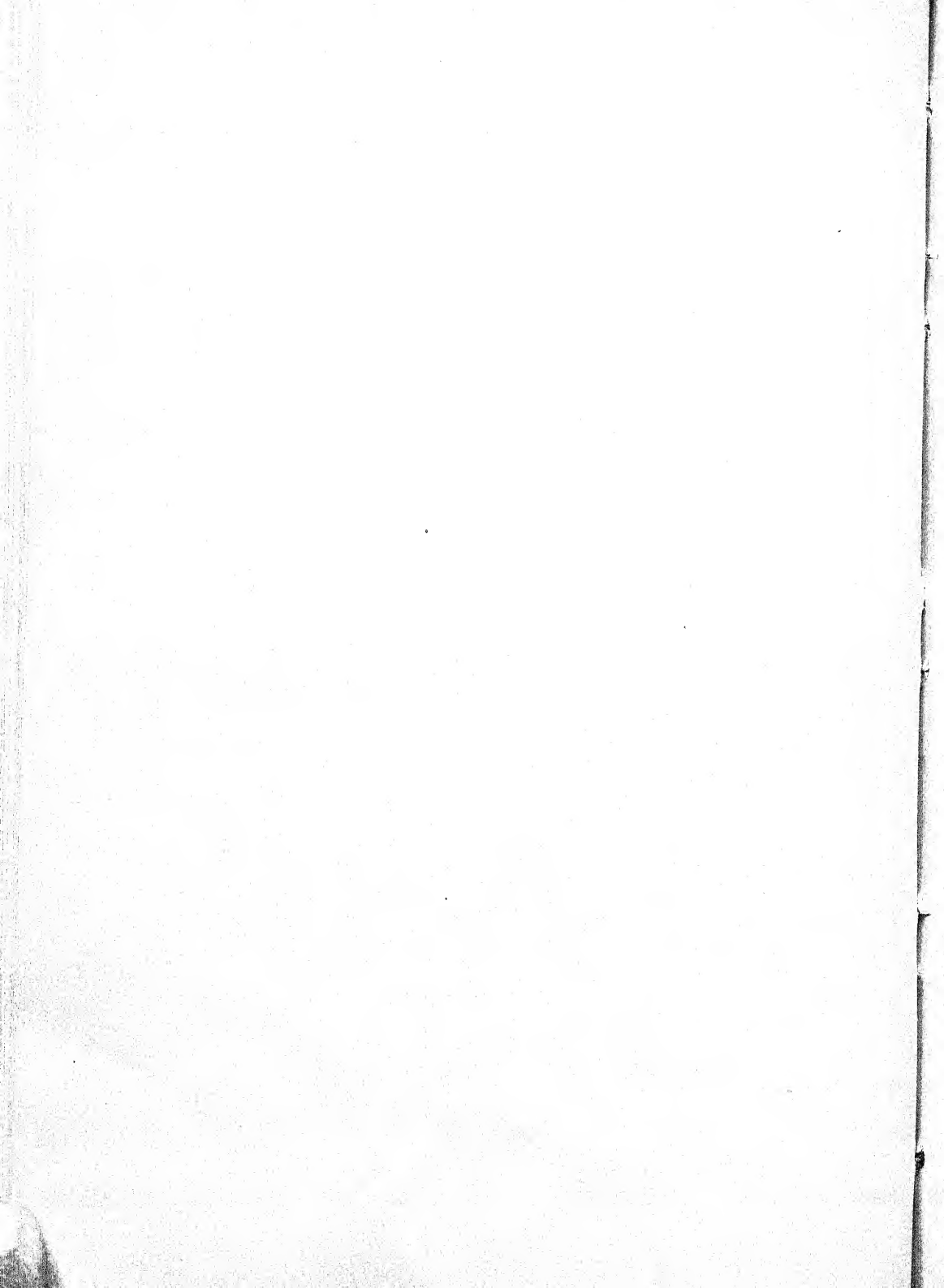


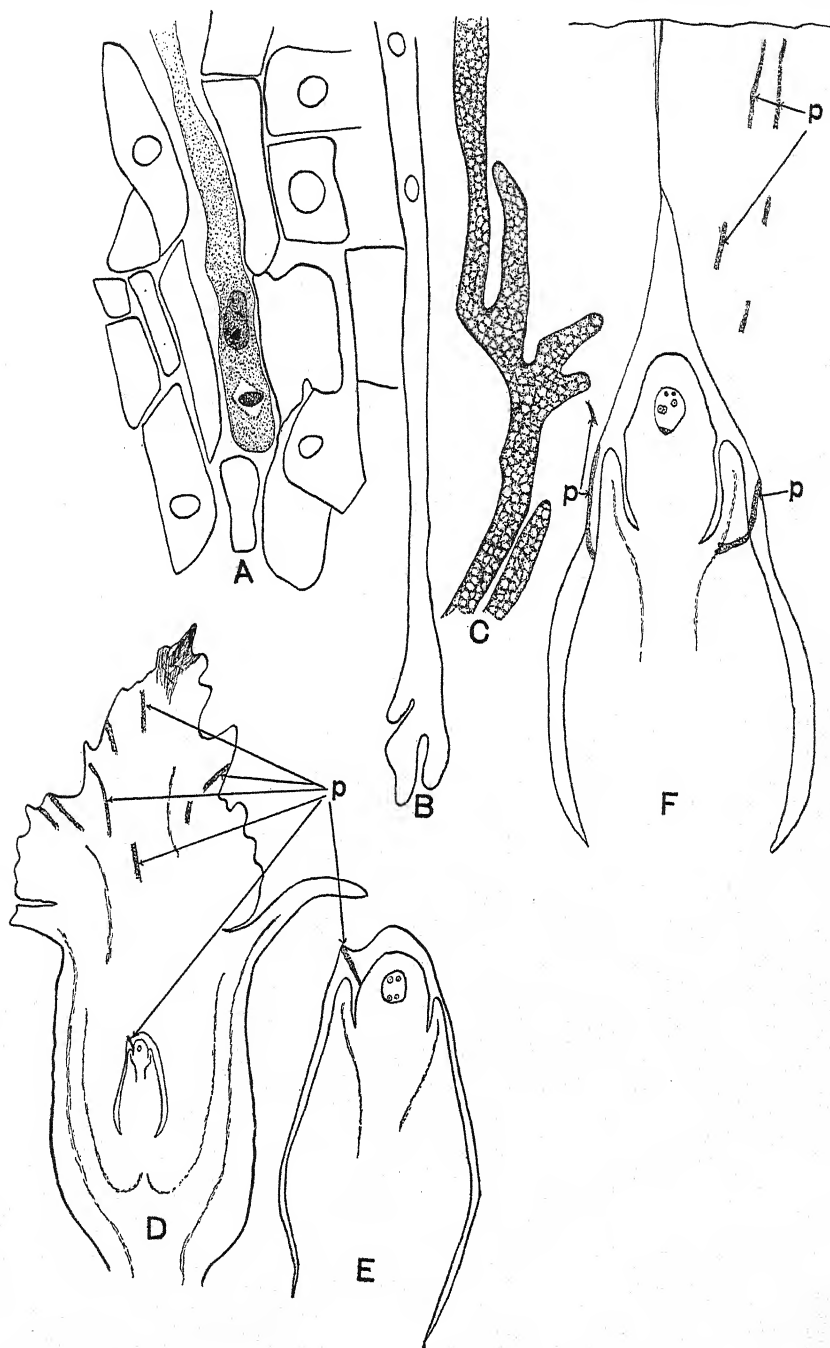
WOODROOF: DEVELOPMENT OF HICORIA PECAN



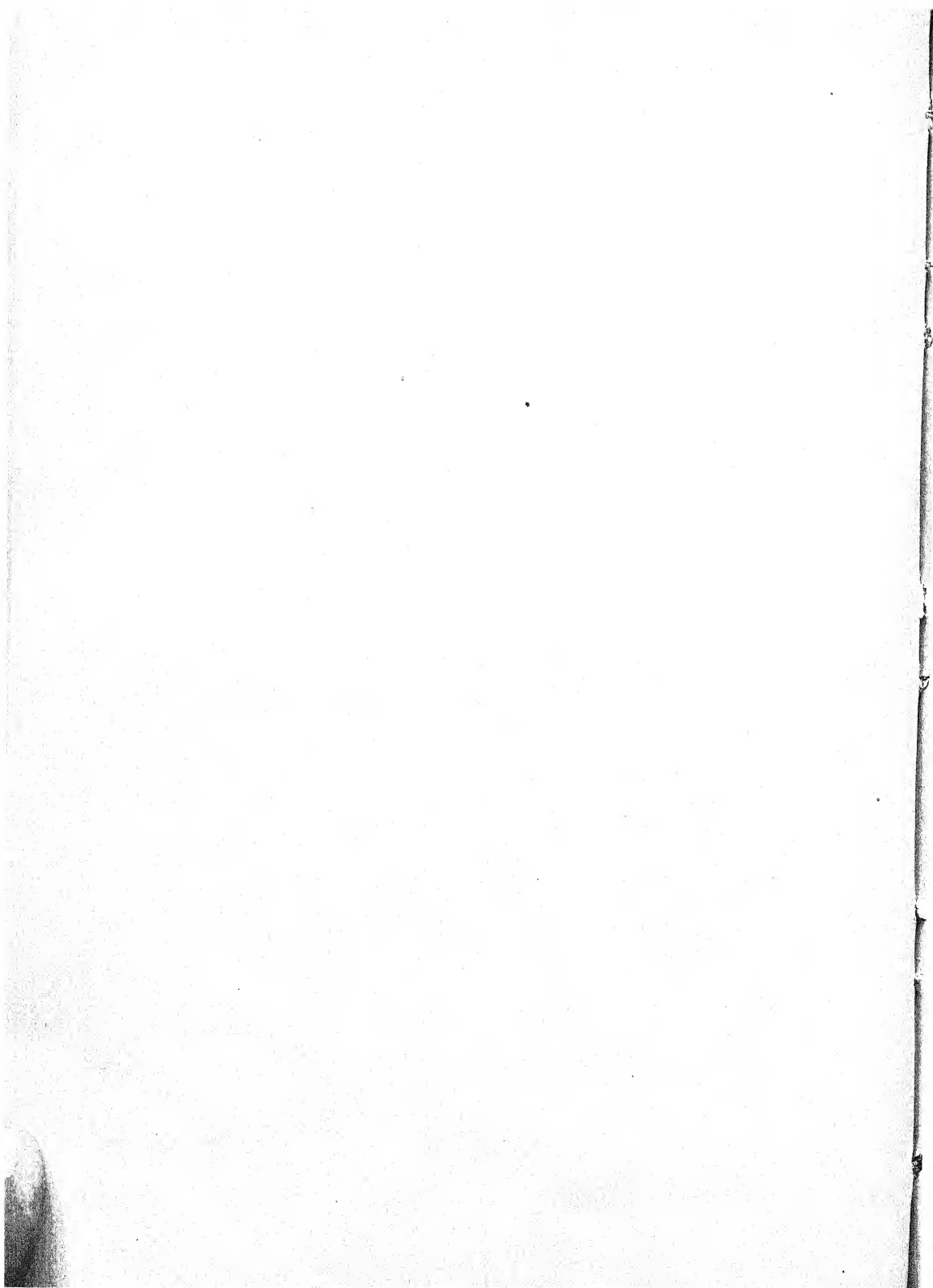


WOODROOF: DEVELOPMENT OF HICORIA PECAN





WOODROOF: DEVELOPMENT OF HICORIA PECAN



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EXPLANATION OF PLATES

Key to abbreviations: *a*, synergid nuclei; *b*, egg nucleus; *c*, polar nuclei; *d*, antipodals; *e*, male nucleus; *p*, pollen tubes.

PLATE XXIII

- FIG. A. Nucellus and integument with a large megaspore mother cell. $\times 72$.
FIG. B. Four megaspores. The large chalazal megaspore is developing. $\times 830$.
FIG. C. One-nucleate embryo sac. Three megaspores have disintegrated to a shapeless mass. $\times 830$.
FIG. D. First nuclear division in the embryo sac. $\times 830$.
FIG. E. Two-nucleate embryo sac after the first nuclear division. Note the two large vacuoles. $\times 830$.
FIG. F. Two-nucleate embryo sac with one large vacuole. Nuclei have migrated to opposite ends of the sac before dividing. Traces of the non-functional megaspores are still visible. $\times 830$.
FIG. G. Mature embryo sac, polar nuclei fusing. About one week after pollination. $\times 830$.
FIG. H. Embryo sac after the fusion of the polar nuclei. About a week to ten days after pollination. $\times 815$.

PLATE XXIV

- FIG. A. End of pollen tube with two male nuclei (*e*), projecting into the embryo sac. About two weeks after pollination. Antipodals have disintegrated. $\times 830$.
FIG. B. Migrated egg nucleus with male nucleus near. About five weeks after pollination. Endosperm lining the embryo sac. $\times 815$.
FIG. C. Fertilized egg with remnants of a pollen tube above and extending into the sac. Seven weeks after pollination. $\times 815$.
FIG. D. Endosperm showing the free nuclei.
FIG. E. Fertilized egg eight weeks after pollination. $\times 830$.
FIG. F. Eight-celled embryo. Five nuclei shown in section. $\times 815$.
FIG. G. Young embryo about ten weeks after pollination. $\times 830$.

PLATE XXV

- FIG. A. Pollen tube growing in stigmatic tissue. $\times 815$.
FIGS. B and C. Branched pollen tubes. $\times 815$.
FIG. D. Pollen tubes (*p*) growing in stigmatic tissue. The tip of the stigma has begun to dry. $\times 16$.
FIG. E. An enlargement of the placenta and ovule shown in D, illustrating a pollen tube approaching the nucellus from above rather than through the chalaza. $\times 27$.
FIG. F. Pollen tubes (*p*) approaching the ovule through the tissue about the stylar canal. Two have entered the crevices and crossed to the placenta. $\times 72$.

THE EFFECT OF ETHYL ALCOHOL ON THE TURGOR-PRESSURE OF *SPIROGYRA*

W. W. LEPESCHKIN

(Received for publication February 4, 1928)

It is well known that ethyl alcohol is a poisonous and, at the same time, narcotic substance. Therefore great concentrations of alcohol must injure protoplasm. In this case alcohol acts in a double manner. First, it accelerates the disintegration of protein substances composing living matter and produces a destruction of the compounds they form with lipoids.¹ Second, taken in sufficiently great concentration alcohol dehydrates colloidal particles of protoplasm. The hydrophilic colloids of protoplasm are transformed by this dehydration into hydrophobic ones, and coagulation occurs. It acts mechanically on the compounds of protein substances and lipoids and brings about their decomposition, also.

On the other hand, every decomposition of the principal substances of protoplasm (lipo-proteids) diminishes the amount of lipoids in its dispersion medium because free lipoids arising by the decomposition form dispersed phases. Therefore the permeability of protoplasm must increase from the effect of great concentrations of alcohol.

Weak concentrations of alcohol produce anaesthesia. One supposes that this state is brought about by the accumulation of narcotic substances in protoplasm. Therefore the accumulation of alcohol must produce a decrease of the permeability of protoplasm for salts, sugar, and other substances insoluble in alcohol.

Turgor-pressure is mainly osmotic pressure, but the osmotic pressure in a cell depends not only upon the concentration and temperature of cell sap but also upon the permeability of protoplasm for substances dissolved in the cell sap. The greater the permeability, the smaller is the osmotic pressure observed in the cell. Therefore we should expect that strong concentrations of alcohol would diminish the turgor-pressure of the cells of *Spirogyra*, while weak concentrations of alcohol would increase it; but this supposition proved to be wrong. My experiments have shown that weak concentrations of alcohol decrease the turgor-pressure while strong concentrations of it increase this pressure. If the concentration of alcohol was 30 to 50 percent the increasing effect on the turgor-pressure of *Spirogyra* was so great that the filaments of the alga were torn to pieces and the cells burst. Too great concentrations of alcohol did not produce this phenomenon because they killed the cells instantaneously.

¹ Lepeschkin, W. W. Kolloidchemie des Protoplasma. Berlin, 1924.

The measurement of the turgor-pressure was made by the method described by me several years ago.² This method is based on the fact that a given extension of cell wall corresponds with a given turgor-pressure in the cell. The change of extension can be determined by the exact measurement of the change of the length of *Spirogyra* filaments. My preliminary experiments showed that dilute alcohol does not alter the mechanical properties of cell walls. Furthermore, the growth of the alga proved to influence the length of *Spirogyra* only very insignificantly during the experiment.

The results obtained are shown in table I. The length of filaments of *Spirogyra* (L) is expressed in divisions of an object-micrometer (each division = 1/75 centimeter). L_0 is the length of the same filaments after the disappearance of turgor-pressure (the cells being killed by iodine and potassium iodid). P is the turgor-pressure expressed in atmospheres. $\frac{L - L_0}{L_0}$ is the relative extension of the cell walls due to turgor-pressure. The exactitude of the measurement of length was 0.005 division of the object-micrometer, or about 1 μ .

TABLE I.

Experiment No.	Liquid Surrounding <i>Spirogyra</i>	L	$\frac{L - L_0}{L_0}$	P
I.....	Water	66.62	0.0424	12.8
	2% alcohol	66.42	0.0392	8.6
II.....	Water	59.19	0.0444	13.5
	1% alcohol	59.17	0.0439	12.1
III.....	Water	59.15	0.0387	11.2
	10% alcohol	59.20	0.0396	14.5
IV.....	Water	65.06	0.0311	12.5
	18% alcohol	65.21	0.0335	16
V.....	25% alcohol	The filaments were broken into three pieces; one cell burst.		
VI.....	35% alcohol	The filaments were broken into nine or more pieces; many cells burst.		

The cells of *Spirogyra* burst, in strong alcohol, usually by one opening; sometimes only the cells situated at the ends of the filaments burst to the outside, while the other cells burst into the inside of these cells through an opening in the partition wall.

Further experiments showed that the increasing effect of stronger concentrations of alcohol upon the turgor-pressure of *Spirogyra* must be ascribed to a very strong rise of the concentration of cell sap of this alga. Its cell sap contains much tannin, which forms a colloidal solution in water, but the solutions of tannin in diluted alcohol are molecular. Therefore alcohol penetrates into the cell sap, dissolves tannin molecularly, and increases its molecular concentration.

² Lepeschkin, W. W. Zur Kenntnis des Wachstumsmechanismus. Beih. Bot. Centralbl. 1907.

Concerning the decreasing effect of small concentrations of alcohol on the turgor-pressure of *Spirogyra*, the method of isotonic coefficients showed that this is produced by the increase of the permeability of protoplasm. Such a result proves once more that not all narcotics lower permeability because narcosis is brought about first of all by a slight chemical decomposition and coagulation of living matter. In my two last papers³ was reported the fact that a slight decomposition of the principal substances of protoplasm, and even a slight coagulation in protoplasm, is often harmless for the cell. The decrease of permeability produced by the accumulation of alcohol is therefore smaller than the increase brought about by the destruction of lipo-proteids of protoplasm and by the diminution of lipoids in the dispersion medium.

³ **Lepeschkin, W. W.** Zur Kenntnis der mechanischen Koagulation des Protoplasma. Arch. Exper. Zellforschung 1927; Über Zusammenhang zwischen mechanischen und chemischen Schädigungen des Protoplasma. Protoplasma 2, 1927. See also **Weber und Hofer.** Permeabilität des Harnstoffs. Jahrb. Wiss. Bot. 1926.

GENERA OF NORTH AMERICAN FABACEAE
IV. TRIBE PSORALEAE (CONTINUED)

P. A. RYDBERG

(Received for publication February 15, 1928)

9. *Apoplanesia* Presl. Symb. Bot. 1: 63. 1831

Large shrubs or small trees, with glandular-dotted foliage. The leaves are alternate, odd-pinnate, but the leaflets are usually more or less alternate; stipules are lacking. The flowers are borne in axillary and terminal panicles. The calyx has a short tube and 5 slightly unequal, foliaceous lobes, reticulate, 3-ribbed, obtuse, accrescent in fruit. The corolla is sub-papilionaceous, the petals short-clawed, nearly equal in length, all free; the upper petal or banner is obovate, somewhat reflexed, the rest are similar, oblong-ob lanceolate, somewhat oblique. The stamens are 10, monadelphous at the base, the staminal tube short, split on the upper side. The ovary is sessile, with 1 or 2 ascending ovules; the style filiform, persistent, hooked at the apex, the stigma capitate. The pod is coriaceous, indehiscent, compressed, suborbicular, conspicuously glandular-punctate, mostly 1-seeded.

ILLUSTRATION: Plate XXVI I. *Apoplanesia paniculata* Presl. $\times 2/3$; 1. calyx, side-view; 2. calyx, front-view, with fruit; 3. banner; 4. wing; 5. keel-petal, $\times 2$; 6. stamens; 7. pistil, $\times 3$; 8. cross-section of pod; 9. seed, $\times 2$.

The genus was based on a single species, *A. paniculata* Presl.

SYNONYM:

Microlobium Liebm. Vidensk. Meddel. 1853: 104. 1854. It was based on *M. glandulosum* Liebm., which is the same as *A. paniculata*.

The genus consists of two species, the type species from Mexico and *A. cryptopetala* Pittier from Venezuela. The genus had been placed with the first group of genera, which is characterized by the single ovule. In reality it is more closely related to the second group with more than 1 ovule. The closest affinity is perhaps with *Amorpha*. The existence of a second ovule in the Venezuelan species also indicates such a relationship. On account of the slightly developed zygomorphy of the flower and the accrescent sepals, this genus should perhaps deserve a distinct group by itself.

10. *Parryella* T. & G.; A. Gray, Proc. Am. Acad. 7: 397. 1868

Low shrubs, with glandular-punctate foliage. The leaves are alternate, odd-pinnate with many entire leaflets. The flowers are borne in terminal racemes. The calyx is turbinate, 10-striate, with 5 short equal teeth, strongly glandular-punctate. A corolla is wanting. The stamens are 10; the filaments free except at the base, inserted in the hypanthium; the

anthers are uniform. The ovary has 2 ovules; the style is thick, bent upwards; the stigma is glandlike, lateral. The pod is indehiscent, obliquely ovoid, conspicuously punctate. The seed is usually solitary, oval, somewhat compressed.

ILLUSTRATION. Plate XXVI J. *Parryella filifolia* T. & G. $\times 2/3$; 1. flower; 2. calyx; 3. stamens; 4. pistil; 5. pod; 6. cross-section of pod; 7. seed, $\times 6$.

The genus was described from a single species, *P. filifolia* T. & G. It consists of two species, natives of Arizona and New Mexico.

It is closely related to *Amorpha*; the reduction of the parts of the corolla has gone a step further so that even the banner is lacking.

11. *Amorpha* L. Sp. Pl. 713. 1753

Shrubs with glandular-dotted foliage. The leaves are odd-pinnate, with entire leaflets and setaceous stipules. The flowers are borne in spike-like racemes or panicles, with small subulate deciduous bracts. The calyx is turbinate, mostly oblique, 5-lobed. The corolla consists of a single petal, the banner, which is erect, incurved, obovate or cuneate in outline, with a more or less developed claw, and folded around the stamens; blue, purple, or white. The stamens are 10, the filaments united at the base only, or one of them wholly free. The ovary is 2-ovuled; the style slender and the stigma terminal. The pod is short, 1- or 2-seeded indehiscent, oblique, somewhat compressed, strongly glandular-punctate, rounded and broader at the apex, the upper suture straight or upcurved. The seeds are oblong and somewhat curved.

ILLUSTRATION: Plate XXVI K. *Amorpha fruticosa* L. $\times 2/3$; 1. flower, $\times 2$; 2. calyx; 3. banner, $\times 4$; 4. stamens; 5. pistil; 6. pod; 7. cross-section of pod; 8. seed, $\times 2$.

The genus was based on *A. fruticosa* L. It consists of 23 known species, all natives of North America. It is characterized by the single petal (banner) and the indehiscent pod.

12. *Eysenhardtia* H. B. K. Nov. Gen. & Sp. 6: 489. 1824

Shrubs or trees, usually much branched. The leaves are alternate, odd-pinnate, with oblong to elliptic, glandular-punctate leaflets. The flowers are borne in loosely clustered terminal spikes or racemes. The calyx is turbinate, strongly glandular-punctate, unequally 5-lobed; the lowest lobe longest, the tube deeper cleft between the upper lobes. The corolla is only slightly zygomorphic, the banner is slightly larger than the other petals, truncate or notched at the apex; the other petals are slightly oblique and those of the keel narrower and distinct. The stamens are 10, diadelphous or nearly so, the upper filament distinct or slightly united to the tube at the base. The ovary is sessile, the style slender, upcurved, bearing a gland near the tip or sometimes straight and glandless; the stigma capitate. The pod is indehiscent, but not of a very thick texture, unlike the rest of the group in that respect; the ovules are 2-4, but usually only one pendulous seed is developed.

ILLUSTRATION: Plate XXVI L. *Eysenhardtia polystachya* $\times 2/3$; 1.

calyx; 2. banner; 3. wing; 4. keel-petal, $\times 2$; 5. stamens; 6. pistil, $\times 4$; 7. pod; 8. cross-section of pod; 9. seed, $\times 2$.

The genus was based on a single species, *E. amorphoides* H. B. K., which is the same as *E. polystachya* (Ortega) Sarg.

SYNONYMS:

Viborquia Ortega, Dec. 66. 1798. The type of the genus was *V. polystachya*, which is the same as *E. amorphoides* H. B. K. The genus was named in honor of "D. Viborq" of Copenhagen, a misspelling of Viborg. The name is therefore preoccupied by *Viborgia* Moench, 1794. O. Kuntze changed the spelling to *Wiborgia*.

Varennea DC. Prodr. 2: 522. 1825. This was a substitute for the untenable *Viborquia* and hence had the same type.

Wiborgia Kuntze, Rev. Gen. 213. 1891. See *Viborquia*.

The genus is evidently related to *Amorpha*, but all 5 petals are present, the texture of the pod is membranous rather than coriaceous, and the 9 filaments are united half their length. It consists of 14 species, natives of southwestern United States, Mexico, and Guatemala.

13. *Psorobatus* Rydb. N. Am. Fl. 24: 40. 1919

Low shrubs, with spinescent stipules and glandular-pustulate branches. The leaves are alternate, odd-pinnate with orbicular leaflets. The flowers are borne in elongate spikes. The calyx is deeply campanulate, almost regular, with oblong lobes. The petals are yellow, somewhat fleshy, distinct, scarcely oblique and scarcely clawed, the upper petal broader than the rest. The stamens are 9, monadelphous, the alternate ones shorter. The ovary is short, 2-ovuled, the style short, hooked at the apex, bearing a gland at the bend; the stigma is capitate. The pod is ovoid or ellipsoid, turgid, glandular-punctate, with a short slender beak. The seed is usually solitary, oblong.

ILLUSTRATION: Plate XXVII M. *Psorobatus Benthami* (Brand.) Rydb. $\times 2/3$; 1. calyx, side-view; 2. calyx, laid open, $\times 2$; 3. banner; 4. wing; 5. keel-petal; 6. stamens; 7. pistil, $\times 4$; 8. pod; 9. cross-section of pod; 10. seed, $\times 2$.

Type species: *Dalea Benthami* Brand.

The genus consists of 2 species of Lower California and Sonora, originally described as species of *Dalea* (*Parosela*). They can not be included in that genus as the corolla is not papilionaceous, the clawless petals are wholly free from the staminal tube, the keel-petals free from each other, the general habit peculiar, and the stipules spinulose. J. F. Macbride's criticism of my removing them from *Parosela* is therefore unwarranted. They might have been included in the South American genus *Errazurizia* Phil., as I. M. Johnston has done. This disposition was considered by me when the genus *Psorobatus* was established; but then I did not think it best and do not do so now. For the sake of comparison I have included here said genus.

Errazurizia Phil. An. Univ. Chil. 1872. 689. 1872

The genus consists of a single species, *E. multifoliata* (Clos.) Johnston, of Chili. This plant resembles greatly the species of *Psorobatus* in general habit, in the white or gray, tomentose and strongly glandular stems, the strict spike, and the ascending flowers with a corolla that slightly exceeds the calyx; but the stipules are subulate-filiform, somewhat fleshy, not becoming spinescent, the banner is villous within, the other petals are distinctly clawed, decidedly oblique, and those forming the keel broader than the wings and their blades adnate above. The ovary is described as 1-ovuled.

ILLUSTRATION: Plate XXVII N. *Errazurizia multifoliata* (Clos.) Johnston $\times 2/3$; 1. and 2. calyx, $\times 2$; 3. banner; 4. wing; 5. keel-petals; 6. stamens; 7. pistil, $\times 4$.

The type of the genus is *E. glandulifera* Phil. with the following synonyms: *Psoralea multifoliolata* Clos., *Dalea multifoliolata* Phil., *Parosela multifoliolata* Macb., *E. multifoliolata* Johnston. It is evident that it belongs neither to *Psoralea* nor to *Parosela*. The relationship is with *Psorobatus*; whether the latter should be merged in it is a matter of taste. The distinctly clawed and decidedly oblique wings and keel-petals, the united keel-petals, the soft stipules, and the peculiar banner, densely pubescent within, are the distinguishing characters in *Errazurizia*.

14. Psorodendron Rydb. N. Am. Fl. 24: 41. 1919

Shrubs or small trees, with glabrate, gray or straw-colored stems, the branches often spinescent, pubescent and glandular-dotted. The leaves are alternate, glandular-dotted, odd-pinnate or simple. The flowers are borne in racemes or spikes, subtended by subulate bractlets and with a deciduous bract at the base of the pedicel. The calyx-tube is turbinate, the hypanthium slightly adnate to the ovary. The corolla is truly papilionaceous, the petals are inserted on the hypanthium at the base of the staminal tube, distinctly clawed, and (except the banner) distinctly oblique, and with a basal auricle, the blades of the keel-petals slightly adnate along the lower edge. The stamens are 9 or 10, monadelphous. The ovary is mostly 2-ovuled, in one species 4-6-ovuled, pubescent, the style is glabrous and curved, the stigma minute. The pod is more or less compressed, glandular-dotted, mostly 1- or 2-seeded.

ILLUSTRATION: Plate XXVII O. *Psorodendron Johnstoni* (S. Wats.) Rydb. $\times 2/3$; 1. calyx; 2. banner; 3. wing; 4. keel-petal; 5. and 6. stamens; 7. pistil; 8. pod; 9. cross-section of pod; 10. seed, $\times 2$.

The genus was established on 12 species of *Dalea* (or *Parosela*), native of western North America. Among these *Dalea Johnstoni* S. Wats. was selected as the type.

SYNONYM:

Asagraea Baillon, *Adansonia* 9: 232. 1870, with *Dalea spinosa* A. Gray as the type. The name *Asagraea* is not tenable, however, as there is an older *Asagraea* Lindley, 1839.

Dalea § *Xylodalea* S. Wats. Proc. Am. Acad. 11: 132. 1876, as to the larger number of species, belongs also here.

This genus and the next differ from *Parosela* in the insertion of the petals, which is truly basal and not on the staminal tube. It is confined to North America.

15. *Phorothamnus* Rydb. N. Am. Fl. 24: 45. 1917

Intricately branched shrubs, with glandular-pustulate branches, and alternate, odd-pinnate or simple leaves. The flowers are borne in dense, short, often subglobose spikes, the bracts lanceolate, the bractlets wanting or represented by glands. The calyx has a turbinate, strongly 10-ribbed tube, with rows of glands in the intervals. The corolla is dark-blue or dark-purple, papilionaceous; the petals, are inserted at the base of the staminal tube, and all clawed; the blade of the banner suborbicular or round-cordate, that of the other petals obliquely oval, with a rounded basal auricle. The stamens are 9 or 10, monadelphous. The pod is obliquely obovoid, turgid, glandular-dotted, pubescent above, usually 1-seeded.

ILLUSTRATION: Plate XXVII P. *Psorothamnus Emoryi* (A. Gray) Rydb. $\times 2/3$; 1. calyx, $\times 2$; 2. banner; 3. wing; 4. keel-petal, $\times 4$; 5. and 6. stamens; 7. pistil, $\times 2$; 8. pod of *P. scoparius* (A. Gray) Rydb.; 9. cross-section of pod; 10. seed, $\times 2$.

The genus was based on 8 West American species of *Dalea* (*Parosela*), of which *Dalea Emoryi* A. Gray was taken as the type.

16. *Parosela* Cav. Descr. Pl. 185. 1802

Annual or perennial herbs, or shrubs, with more or less glandular-dotted calyces, leaves, and branches. The leaves are alternate, odd-pinnate, with stipule and stipels, both often gland-like. The flowers are borne in racemes or spikes. The calyx-tube is campanulate or turbinate, 10-ribbed and 5-lobed, the lowest lobe often much longer. The banner is inserted at the base of the staminal tube, long-clawed, commonly with a cordate or reniform blade, the wings and keel-petals are short-clawed or sessile, inserted on the staminal tube more or less high up, but mostly below the middle, the keel-petals higher up than the wings; the blades are usually obliquely obovate, usually with a distinct basal auricle, those of the keel-petals usually broader than those of the wings and usually united towards the tip. The stamens are usually 10, or 9, monadelphous, the tube split on the upper side. The ovary is 1- or 2-ovuled, the style nearly straight and the stigma capitate. The pod is indehiscent, obliquely obovoid, or semi-reniform, included in the calyx, usually 1-seeded.

ILLUSTRATION: Plate XXVIII Q. *Parosela mutabilis* Cav. $\times 2/3$; 1. calyx, $\times 3$; 2. androecium and 4 petals from a young flower; 3. banner from the same, $\times 1$; 4. banner; 5. wing; 6. keel-petal; 7. and 8. stamens, with the position of the insertion of the petals indicated; 9. pistil, $\times 2$; 10. pod; 11. cross-section of the pod; 12. seed, $\times 4$.

The genus was established with *Psoralea mutabilis* Cav. as the type.

SYNONYMS:

Dalea Vent. Tab. Veg. 3: 396, in part. 1799. Not *Dalea* Miller 1754. Ventenat revived an old generic name, used before 1753, and included seven species of *Psoralea* of various authors. Of these all belong to this genus except *Psoralea Dalea* L., which should be regarded as the type. *Cylopogon* Raf. Jour. Phs. 89: 97. 1819. This genus was based on two species, *C. capitatum* and *C. virgatum*, of which the first may be regarded as the type. It is the same as *P. aurea*.

Jamesia Raf. Atl. Jour. 145. 1832. *J. obovata* Raf., which is the same as *P. Jamesii*, as the only species.

Trichopodium Presl. Bot. Bemerk. 52. 1844. This was based on *T. glandulosum* Presl. The author also included *T. diffusum* (*Dalea diffusa* Moric.). The generic name is, however, antedated by *Trichopodium* Lindley. 1832.

Marina Liebm. Vidensk. Meddel. 1853: 103. 1853. This was based on a single species, *M. gracilis*, which is the same as *Parosela delicata* Rose.

Carroa Presl. Symb. Bot. 2: 25. 1858. This was a substitute for the untenable *Trichopodium*.

The genus consists of 180 species, natives of North and Central America, and perhaps 30 more of South America. The North American species may be divided into two subgenera. 1. TRICHOPODIUM, with pedicelled flowers, and 2. EUPAROSELA with sessile flowers. The former comprised as synonyms the genera *Trichopodium*, *Marina*, and *Carroa*. The characters by which it is distinguished from *Parosela* proper are, however, not generic. The original *Trichopodium*, for which *Carroa* was a substitute, represents the typical part of this subgenus. *Marina gracilis* Liebm. differs from the rest, practically, only in the toothed calyx-lobes. *Cylopogon* and *Jamesia* have no generic characters distinguishing them from *Parosela* proper. *Dalea* antedates *Parosela* but is itself antedated by *Dalea* Miller 1754. Furthermore *Dalea*, as a fabaceous genus, was established in 1789 by Jussieu with *Psoralea Dalea* L. as the type; this I regard as belonging to *Thornbera* instead of *Parosela*. *Dalea* as modified by Ventenat is therefore as a synonym of *Parosela*, but only in part.

The subgenus TRICHOPODIUM is represented in North America by 46 species divided into 9 natural groups; the subgenus EUPAROSELA of 134 species in 20 groups.

17. *Thornbera* Rydb. Jour. N. Y. Bot. Gard. 20: 66. 1919

Annual or perennial herbs, with glandular-dotted branches, leaves and calyces. The leaves are alternate, odd-pinnate, with stipules and stipels, the latter glandlike. The flowers are borne in dense spikes. The calyx-tube is campanulate, 10-ribbed and 5-lobed. The banner is inserted in the bottom of the calyx, long-clawed; the blade is ovate or cordate; the other petals are distinct, oblong or oblanceolate, sub-sessile or short-clawed, inserted at the mouth of the staminal tube. The stamens are

9 or 10, monadelphous, the filaments are free above the insertion of the petals. The ovary is sessile, 2-ovuled, the style filiform; the stigma minute. The pod is enclosed in the calyx, 1-seeded, somewhat compressed.

ILLUSTRATION: Plate XXVIII R. *Thornbera albiflora* (A. Gray) Rydb. $\times 2/3$; 1. calyx, $\times 4$; 2. androecium with 4 petals; 3. banner, $\times 2$; 4. banner; 5. wing; 6. keel-petal; 7. and 8. stamens; 9. pistil, $\times 2$; 10. pod; 11. cross-section of pod; 12. seed, $\times 4$.

SYNONYM:

Dalea Juss. Gen. 355. 1789. This was based on *Psoralea Dalea* L. It is antedated by *Dalea* Mill. 1754.

The genus was based on *Dalea albiflora* A. Gray as the type. It consists of 13 species of the southwestern United States, Mexico, and Central America. One species extends into northern South America and is introduced in the Philippine Islands. While the genus was established on species of *Parosela*, the genus is intermediate between that genus and *Petalostemon*, but closer to the latter, from which it differs mainly in the 10 instead of 5 stamens.

18. *Petalostemon* Michx. Fl. Bor. Am. 2: 48. 1803

Perennial or rarely annual glandular-dotted herbs. The leaves are alternate, odd-pinnate with stipules. The flowers are borne in terminal dense spikes, with deciduous bracts. The calyx-tube is campanulate or turbinate, 10-nerved and 5-lobed. The banner is long-clawed, inserted in the bottom of the calyx, with a blade broader than that of the other petals, cordate or truncate at the base; the other petals short-clawed, inserted at the mouth of the staminal tube and alternating with the free portion of the filaments, the blades being narrow and acute at the base. The stamens are 5, monadelphous. The ovary is 2-ovuled, the style filiform, the stigma minute. The pod is membranous, enclosed in the calyx, 1- or 2-seeded, compressed, obliquely obovate.

ILLUSTRATION: Plate XXVIII S. *Petalostemon candidus* (Willd.) Michx. $\times 2/3$; 1. flower, $\times 2$; 2. calyx; 3. androecium, with 4 petals; 4. and 5. banner; 6. wing; 7. keel-petal; 8. pistil; 9. pod; 10. cross-section of pod; 11. seed, $\times 4$; 12. leaflet from the lower leaves, $\times 1$.

The genus was established on 4 species, of which *P. candidus* Michx., being the first and illustrated, has been regarded as the type. It contains 42 species, all North American.

19. *Kuhnistera* Lam. Encyc. 3: 370. 1789

Perennial herbs, with glandular-dotted foliage. The leaves are alternate, odd-pinnate. The flowers are borne in corymbosely arranged head-like spikes, subtended by 3-4 series of persistent, empty bracts. The calyx is deeply cleft, the tube short, turbinate, the lobes subulate-filiform, plumose. The corolla is nearly regular, the petals have oblong blades, the banner is long-clawed and inserted in the bottom of the calyx, the other 4 petals short-clawed and inserted at the mouth of the staminal tube and alternate with the filaments. The stamens are 5, monadelphous, the filaments united half their length. The ovary is sessile, 1- or 2-ovuled, the style filiform, the stigma minute. Pod indehiscent, 1-seeded, densely pubescent.

ILLUSTRATION: Plate XXVIII T. *Kuhnistera pinnata* (Walt.) Kuntze $\times 2/3$; 1. calyx, $\times 2$; 2. androecium with 4 petals; 3. banner; 4. wing; 5. keel-petal; 6. pistil; 7. pod; 8. cross-section of pod; 9. seed.

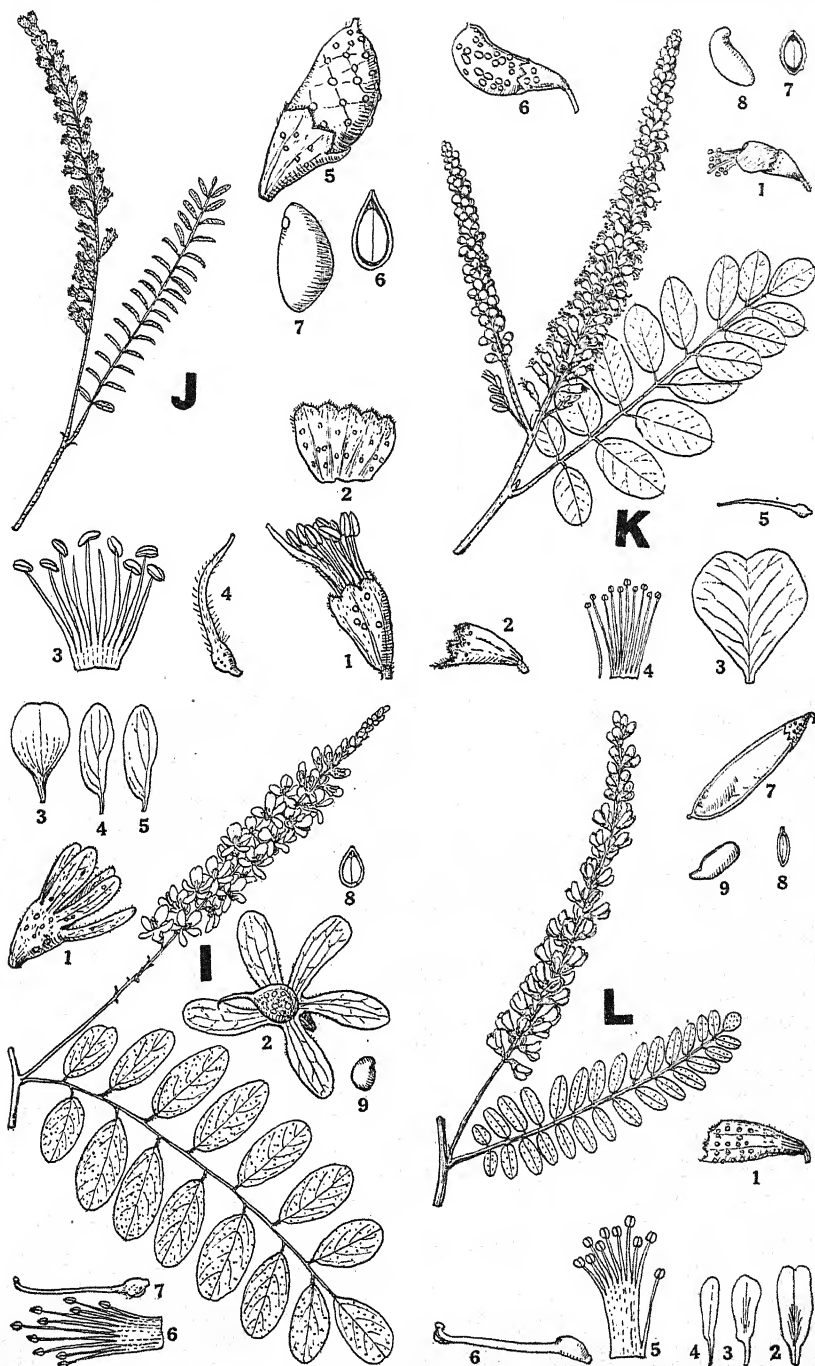
The genus was based on *Kuhnistera caroliniensis* Lam., which is the same as *K. pinnata*.

SYNONYM:

Gatesia Bertol. Novi Comm. Bonon. 9: 212. 1849. Type *G. alabamensis* Bertol., which is the same as *K. pinnata*.

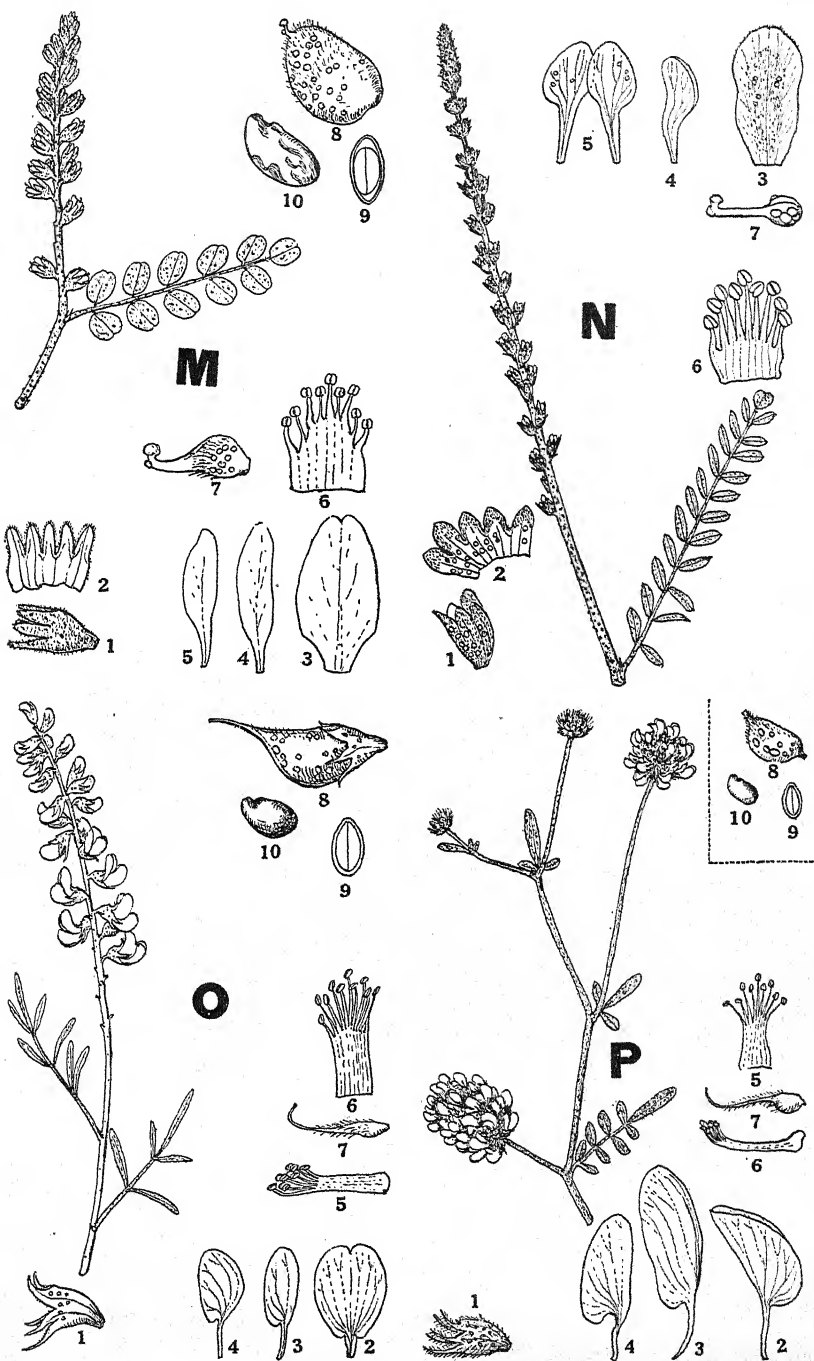
The genus consists of three species of the southeastern United States. It has often been united with *Petalostemon* and the enlarged genus should then bear the older name *Kuhnistera*, but the conspicuous involucre of empty persistent bracts and the long plumose calyx-lobes of *Kuhnistera* show a distinct variation, well worth generic distinction.

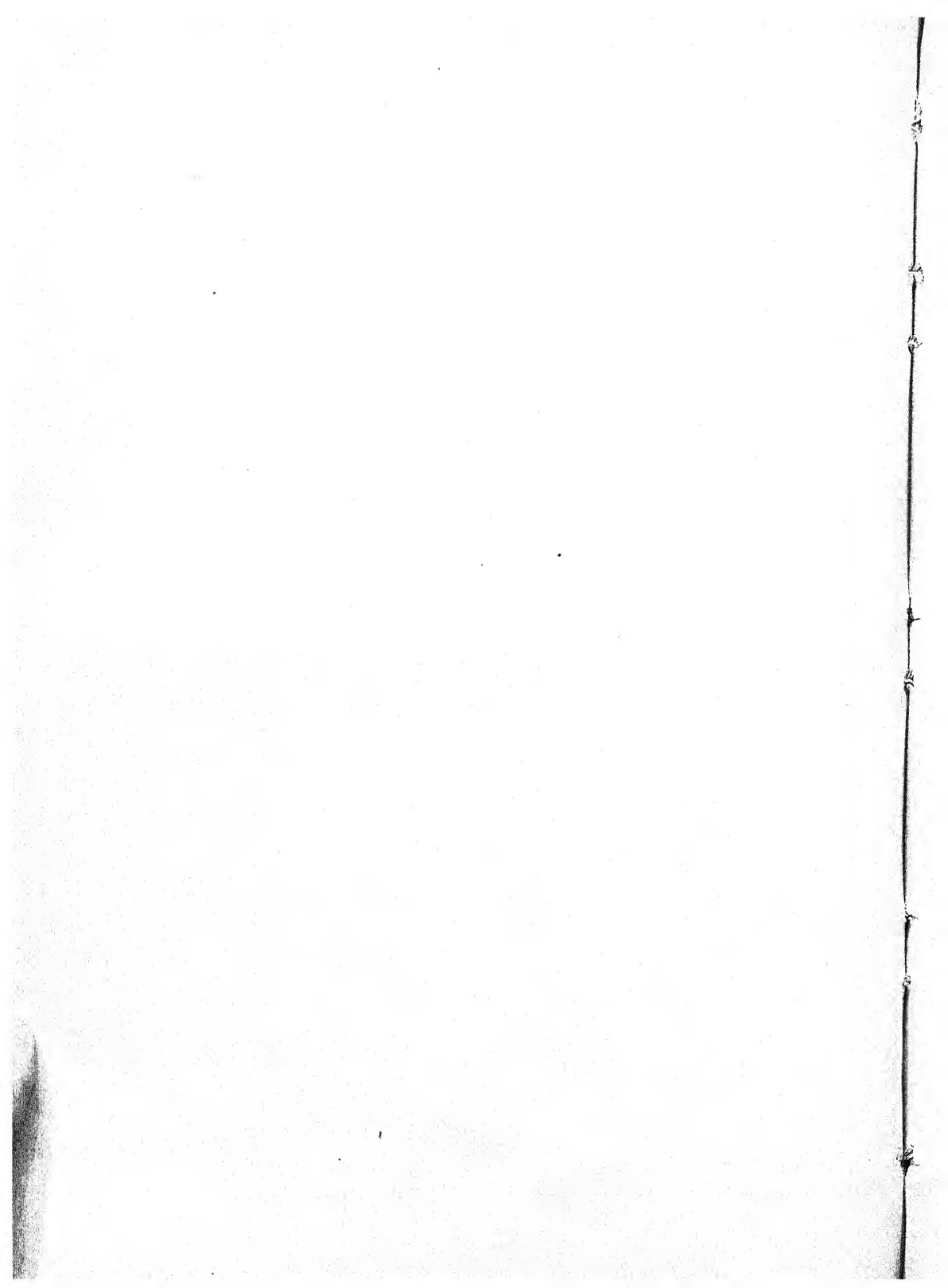
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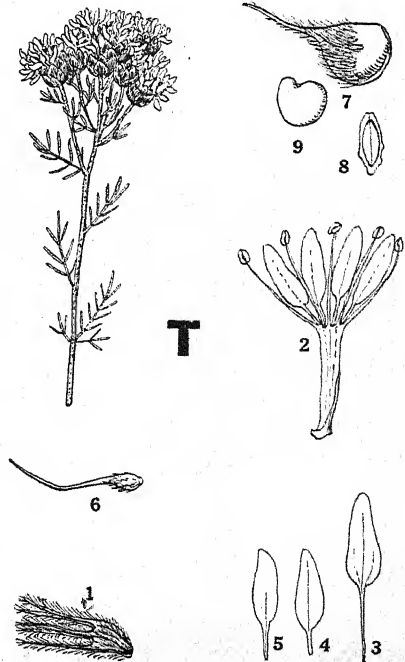
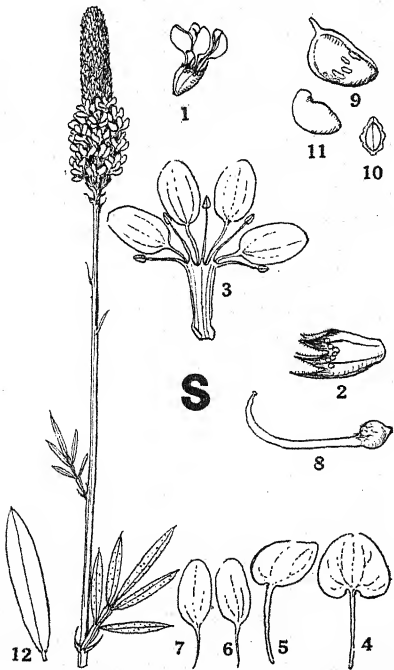
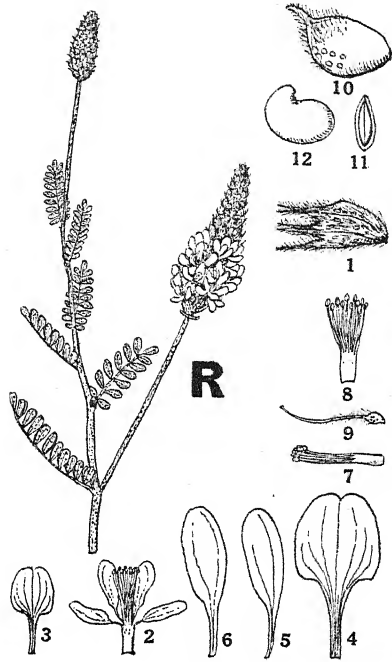
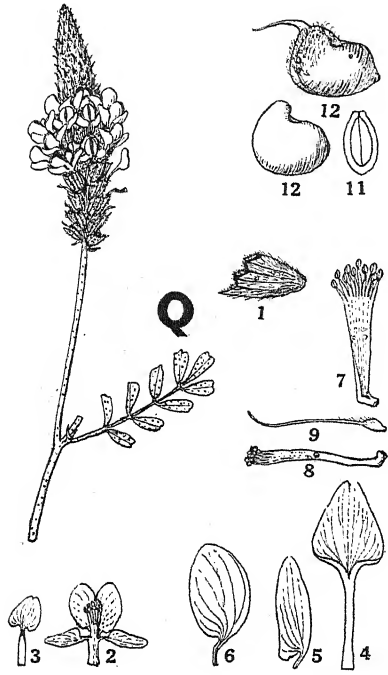


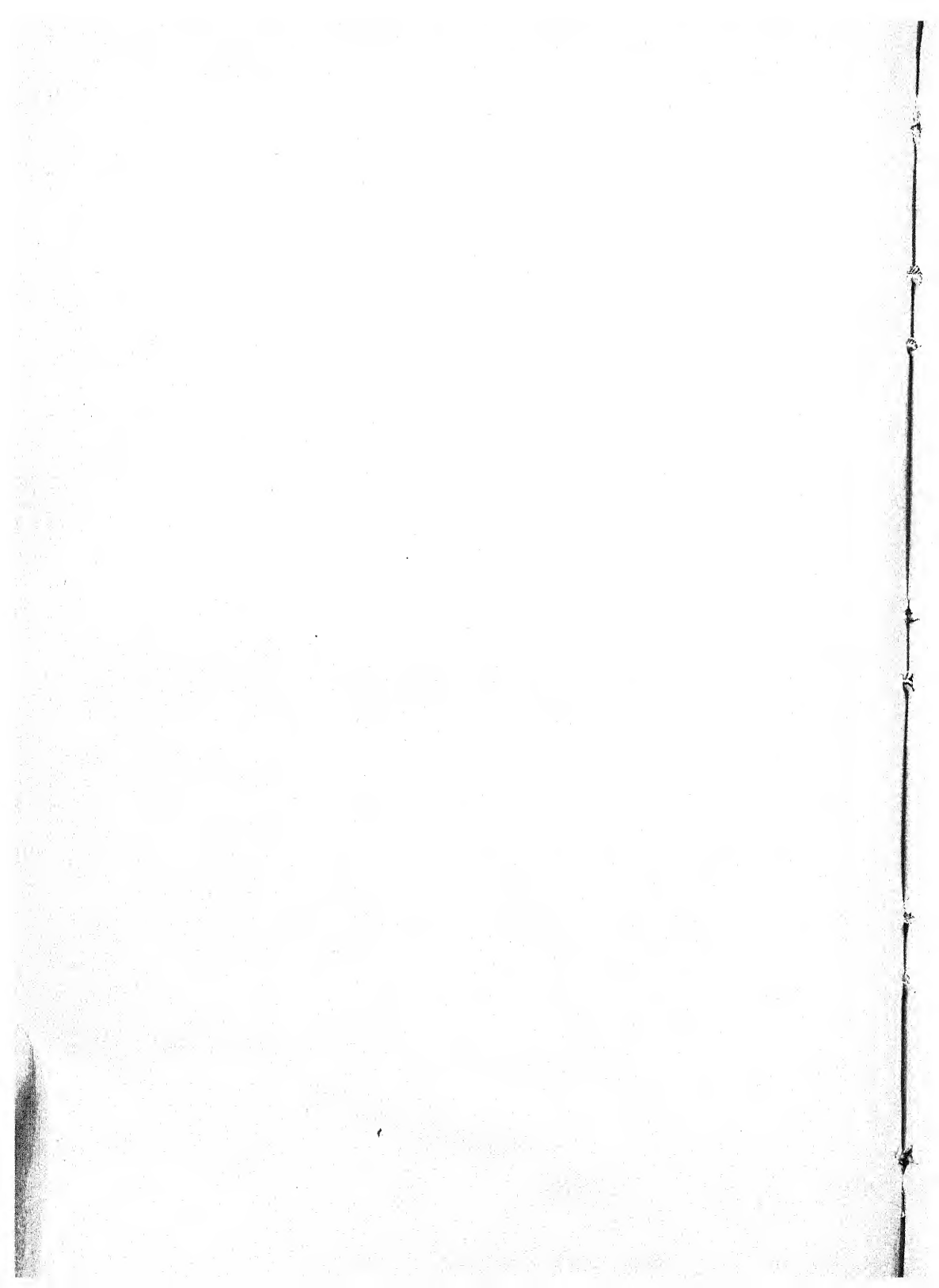
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THE EFFECT OF HIGH PRESSURE ON THE PERCENTAGES OF SOFT AND HARD SEEDS OF *MEDICAGO* *SATIVA* AND *MELILOTUS ALBA*

P. A. DAVIES

(Received for publication March 3, 1928)

INTRODUCTION

In earlier papers^{1, 2} the literature covering certain chemico-physical effects of high pressure and the direct effect of high pressure on germination has been discussed. This paper is concerned with the effect of high pressure on the percentages of soft and hard seeds and the percentage germination of *Medicago sativa* and *Melilotus alba*.

The failure of seeds of *Medicago sativa* and *Melilotus alba* to germinate is caused by two conditions: (1) the loss of vitality by the young embryo (resulting in soft seeds), and (2) the impermeable nature of the seed-coat (resulting in hard seeds). Tests of newly harvested seeds show low percentages of soft seed and high percentages of hard seed.

RESULTS

The results are best described by discussing the data given in each table, comparing the tables, and summarizing the tests as a whole.

Table I shows the effect of a pressure of 2000 atmospheres on germination and percentages of soft and hard seed of *Medicago sativa*. In tests³ made immediately after the pressures were applied, the percentage of soft seed increased rapidly as the duration of the pressure increased, and was 477.74 percent greater in the 10-minute exposure than in the 1-minute exposure. The increases in percentage of soft seed, particularly in the 5- and the 10-minute exposures, resulted in lower percentage germination, indicating that the embryos were either killed or weakened to such an extent (resulting in soft seeds) that they were unable to respond to optimum conditions for germination. Comparing the results of the 1-minute exposure with the results from the average test of the controls,⁴ it will be observed that in

¹ Davies, P. A. Effect of high pressure on germination of seeds (*Medicago sativa* and *Melilotus alba*). Jour. Gen. Physiol. 9: 805-809. 1926.

² —. High pressure and seed germination. Amer. Jour. Bot. 15: 149-156. 1928.

³ Each test represents the average of two germination tests from the same treated bulk. All seeds were germinated on moist filter paper in sterile petri dishes in a germinator at 20° C.

⁴ The controls were untreated seeds from the same bulks as the treated seeds, saved and germinated at the same time as the treated seeds.

TABLE 1. *The Effect of a Hydraulic Pressure of 2000 Atmospheres at $18^{\circ} \pm 2^{\circ}$ C. on Germination and the Percentage of Soft and Hard Seed in Medicago sativa*

Duration of the Exposure, Minutes	Tested Immediately after the Pressure was Applied			Tested after 30 Days			Tested after 6 Months		
	Percentage Germination	Percentage Soft Seeds	Percentage Hard Seeds	Percentage Germination	Percentage Soft Seeds	Percentage Hard Seeds	Percentage Germination	Percentage Soft Seeds	Percentage Hard Seeds
1.....	82.53	10.74	6.73	75.32	15.12	9.56	81.40	11.01	7.59
2.....	72.46	17.85	9.69	79.23	12.92	7.85	76.43	17.75	5.82
5.....	58.90	32.65	8.45	80.92	14.12	4.96	77.06	18.56	4.38
10.....	36.86	62.05	1.09	73.55	25.30	1.15	80.19	18.43	1.38

Average test of the controls: percentage germination, 48.22; percentage soft seeds, 7.63; percentage hard seeds, 44.15.

TABLE 2. *The Effect of a Hydraulic Pressure of 2000 Atmospheres at $18^{\circ} \pm 2^{\circ}$ C. on Germination and the Percentage of Soft and Hard Seed in Melilotus alba*

Duration of the Exposure, Minutes	Tested Immediately after the Pressure was Applied			Tested after 30 Days			Tested after 6 Months		
	Percentage Germination	Percentage Soft Seeds	Percentage Hard Seeds	Percentage Germination	Percentage Soft Seeds	Percentage Hard Seeds	Percentage Germination	Percentage Soft Seeds	Percentage Hard Seeds
5.....	75.70	7.95	16.35	78.37	7.51	14.12	74.19	8.90	16.91
10.....	83.40	5.66	10.94	90.83	3.84	5.33	70.18	20.98	8.84
15.....	80.02	5.16	13.92	85.62	4.79	9.59	71.66	20.32	8.02
20.....	81.58	8.18	10.24	76.23	16.61	7.16	66.28	25.69	8.03
30.....	34.07	60.74	5.19	50.81	42.32	6.87	43.82	50.06	6.12

Average test of the controls: percentage germination, 24.04; percentage soft seeds, 1.14; percentage hard seeds, 74.82.

seeds exposed to pressure there is a slight increase in the percentage of soft seed and a large decrease in the percentage of hard seed. The reduction in the percentage of hard seed results in a higher percentage germination. Results from tests made after 30 days show a reduction in the percentages of soft seed in the 2-, 5-, and 10-minute exposures. These reductions in the percentage of soft seed, with corresponding increases in percentage germination, indicate that after drying for 30 days there is a partial recovery from the injury caused by too long exposures at this pressure. Comparing the 6-months and the 30-days tests, the 1- and the 10-minute exposures in the 6-months tests show decreases in percentage of soft seed, accompanied by increases in percentage germination; while the 2- and the 5-minute exposures show increases in percentage of soft seed and decreases in percentage germination. The reduction in the percentage of soft seed in the 10-minute exposure shows that the maximum recovery did not take place until sometime between 30 days and 6 months.

Table 2 shows the effect of a pressure of 2000 atmospheres on germination and percentages of soft and hard seeds of *Melilotus alba*. In tests made immediately after the pressures were applied, the percentages of soft seed were low and nearly the same in the 5-, 10-, 15-, and 20-minute exposures, but the percentage was very high in the 30-minute exposure. The percentage of hard seed shows a general decrease as the duration of the pressure increased. Comparing the above results with those of the average test of the controls, it will be observed that in the seeds exposed to pressure there is an increase, particularly in the 30-minute exposure, in the percentage of soft seed, and a decrease in the percentage of hard seed. The high percentage of soft seed with the low percentage germination in the 30-minute exposure brings these results into line with those already explained (table 1) on the ground that long durations result in injury to the seeds. Results from tests made 30 days after the pressures were applied show a considerable increase in the percentage of soft seed in the 20-minute exposure and a large decrease in the 30-minute exposure. The low percentage of soft seed in the 30-minute exposure, with a corresponding increase in percentage germination, shows a partial recovery from the injury inflicted by the long exposure at this pressure. The low percentages of soft and hard seed in the 10- and the 15-minute exposures result in higher percentage germinations. The 6-months tests show higher percentages of soft seed and lower percentage germinations; indicating that between 30 days and 6 months there is a gradual loss of vitality by the young embryos.

Comparison of the tables shows that in the tests made immediately after the pressures were applied, the optimum duration for the lowest percentage of soft seed was 1 minute for seeds of *Medicago sativa* and 10 minutes for seeds of *Melilotus alba*. The highest percentage of soft seed and the lowest percentage of hard seed occurred in the 10-minute exposure for seeds of *Medicago sativa* and in the 30-minute exposure for seeds of

Melilotus alba. In tests made 30 days after the pressures were applied, the lowest percentage of soft seed occurred in the 2-minute exposure for seeds of *Medicago sativa* and in the 10-minute exposure for seeds of *Melilotus alba*. In the 6-months tests, the seeds of *Medicago sativa* exposed for 2 and 5 minutes and the seeds of *Melilotus alba* in all exposures show higher percentages of soft seed than were shown in the 30-days tests. The decrease in percentages of soft seed as compared with the tests immediately after the pressure was applied shows a partial recovery from the injury caused by too long exposure at this pressure.

The tests as a whole show that seeds of *Medicago sativa* require a shorter duration at a pressure of 2000 atmospheres than seeds of *Melilotus alba* to produce the same results. As a general rule, there is an increase in the percentage of soft seed and a decrease in the percentage of hard seed as the duration of the pressure increased, and in every case the percentage of soft seed was higher and the percentage of hard seed was lower than in the control.

SUMMARY

1. In tests made immediately after the pressures were applied, the optimum duration for the lowest percentage of soft seed was 1 minute for seeds of *Medicago sativa* and 10 minutes for seeds of *Melilotus alba*. The highest percentage of soft seed and the lowest percentage of hard seed occurred in the 10-minute exposure for seeds of *Medicago sativa* and in the 30-minute exposure for seeds of *Melilotus alba*.

2. In the 30-days tests, the lowest percentage of soft seed occurred in the 2-minute exposure for seeds of *Medicago sativa* and in the 10-minute exposure for seeds of *Melilotus alba*. The percentages of hard seed were nearly the same as the percentages obtained in the tests made immediately after the pressures were applied.

3. The 6-months tests, with the exception of the 1- and the 10-minute exposures for seeds of *Medicago sativa*, show higher percentages of soft seed than were shown in the 30-days tests.

4. In every test the percentage of soft seed was higher and the percentage of hard seed lower than in the control.

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A METHOD OF PREPARING THIN CROSS AND
LONGITUDINAL SECTIONS OF COTTON
FIBERS, AND ITS IMPORTANCE
IN CELL-WALL RESEARCH¹

J. KISSER AND D. B. ANDERSON

(Received for publication March 8, 1928)

In recent years there has been a decided revival of interest in the structure of the plant cell wall. Much of this work has been done on the textile fibers, and cotton, particularly, has been extensively studied. In the course of an investigation of certain problems of cotton fiber structure now being made by one of us (Anderson) it became necessary to prepare very thin cross and longitudinal sections of the fibers, in considerable numbers. From such sections it was thought that further light would be thrown upon the physical and chemical relations of the lamellae composing the wall, and also upon the behavior of the spirally wound crystalline fibrillae composing these lamellae.

Cotton fibers, because of their twisted condition, toughness, and hardness, are unusually difficult structures to cut, particularly into such thin, even sections as would be necessary for this work. Largely through the efforts of the senior author (Kisser) a new method of preparing such sections has been found, and since this method offers such promise for cell wall investigations, as well as for other aspects of cotton research, it has seemed advisable to report briefly upon it now.

Using this method we have been able to cut perfect cross sections of cotton fibers as thin as $2\ \mu$ and excellent longitudinal sections $4\ \mu$ in thickness. Apart from the usual sliding microtome, no special apparatus is required and after the preparation of the material hundreds of sections can be cut in a few minutes.

The fibers used in making these sections were of two varieties, Mexican No. 6 and Acala No. 8. They were untreated, apart from ginning, before being used.

Previous attempts to section cotton fibers after imbedding in paraffin have failed, because through dehydration the fibers became so hard and brittle that they were badly torn by the knife. This was particularly true when the knife blade was used in its customary position—perpendicular to the direction of the cutting movement. Our first attempts were also made with the paraffin method and we found that good cross sections as

¹ Papers from the Plant Physiological Institute of the University of Vienna No. 273, of the second series.

thin as $4\ \mu$ could be obtained, if the knife was properly oriented, *i.e.*, so as to lie as nearly parallel to the direction of the cutting movement as sectioning will permit. It was not possible, however, to avoid a rolling of the section, and the unrolling of such delicate sections brought difficulties. These persisted even if the procedure suggested by Kisser² for other material was followed, *i.e.*, in cutting until almost through the paraffin block and then stopping to unroll the section on the knife blade, with a fine brush, before completing the section. The sections are so thin and stick so tightly to the knife blade that they can not be removed without causing some damage. This trouble can be avoided by keeping the knife blade well moistened. Alcohol cannot be used for this purpose since it dissolves slight traces of paraffin; and water, which might seem best suited, cannot be used because of its high surface tension. Water does not spread itself evenly over the surface of the knife blade but collects in drops, which fall off when the knife is moved. This difficulty largely disappears if the surface tension is reduced through the addition to the water of a little gelatine or soap. A solution of 0.1 percent–0.5 percent gelatine is sufficient. This solution spreads readily over the knife and is inactive toward paraffin. When the knife is so moistened, the sections can easily be unrolled with a fine camel's hair brush without danger of their sticking to the blade, and they can readily be transferred from the knife to a slide.³

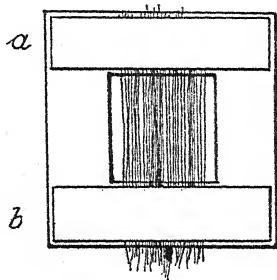
Working with these precautions we were able to prepare good cross sections from paraffin-imbedded material, but the method proved unsuitable for longitudinal sections. Even with the hardest paraffin and with all possible care, the sections were torn and irregular. It was evident that some other method would be necessary in order to prepare thin longitudinal sections. For this purpose, paraffin-celloidin gave very satisfactory results, making possible not only very thin and even longitudinal sections, but also excellent cross sections as thin as $2\ \mu$ without signs of tearing or distortion.

In order to obtain the best sections, the fibers must first be stretched and then imbedded while under tension. Since the fibers are normally colorless and therefore difficult to see after imbedding, it is advisable to stain them with a suitable dye. We used for this purpose carmalum, since most other stains would be removed by the subsequent alcohol-ether treatment. The fibers were first placed in the carmalum solution for a few hours, then thoroughly washed in water, and finally spread out to dry on filter paper. When completely dry, the fibers were combed until parallel and then stretched in cardboard frames. For these frames, pieces of a good strong cardboard 2 cm. square were used. From the center a small window about 8–10 mm. square was cut out and then pressed back into its original position. One end of the cardboard frame was then

² Kisser, J. Leitfaden der botanischen Microtechnik. Jena, 1916.

³ Kisser, J. Zur Anfertigung von Paraffin-Einselschnitten mit schrag gestelltem Messer. Zeit. Wiss. Mikroskopie 45. 1928.

covered with a thin layer of a concentrated solution of gum arabic, and one end of the fiber group laid upon it. The fibers were then uniformly arranged with a needle so that they were evenly distributed over the center window, and this one end was firmly cemented in place. This was done by pressing a narrow strip of cardboard, also covered with a little gum arabic solution, upon the cemented end of the fibers. Only a little gum arabic should be used, since under pressure it will ooze out and cover the fibers, preventing proper imbedding. The pressure should be continued until the gum arabic is firm. The fibers can now be pulled strongly with the fingers or with tweezers, and, while under tension, the other end may also be cemented in a similar way. Until the gum arabic is dry, the tension can still further be increased by pulling on the projecting ends of the fibers and clamping the cardboard strip firmly in place. When the gum arabic is completely dry, the central window can be pushed out, leaving the fibers tightly stretched over the opening (text fig. 1).



TEXT FIG. 1. Fibers mounted for imbedding. At *a* and *b* the fibers are cemented in place with cardboard strips and gum arabic.

After trimming off the projecting fiber ends, the frame with the stretched fibers is placed in 96-percent alcohol for 24 hours. The alcohol is then poured off and a mixture of equal parts of alcohol and ether is added, this also remaining for 24 hours. To insure proper infiltration the fibers are now allowed to stand for about 24 hours each in solutions of 2-percent, 4-percent, and 8-percent celloidin, and then are covered with 8-percent celloidin in a small imbedding dish and the celloidin allowed to thicken. The thickening need not be done in a desiccator; it is sufficient to cover the dish with a glass plate and to place it where it will not be disturbed. The celloidin must not become too hard since this prevents a proper consistency when paraffin is added. When the celloidin has reached the proper degree of hardness—slightly yielding to the fingers—the block should be placed in chloroform and allowed to remain for a few hours to harden the surface. When the surface is hard the block can be taken out, and the center of the frame, with the stretched fibers, carefully cut away. This should be done with a very thin sharp knife, preferably a safety-razor blade.

A thicker blade will mash the celloidin and loosen the fibers. It is better to place the celloidin block so that the cardboard frame lies on the upper side, and cut with a thin blade along the margins of the center window. When this is carefully done the center can easily be removed without disturbing the fibers. The small celloidin block containing the fibers should be placed in a vial of chloroform and allowed to remain until it sinks. In order to remove all traces of alcohol and ether, this should be repeated at least once more. When the block is completely saturated with chloroform, paraffin of 52° melting point is added and the vial placed in the oven. When the chloroform has completely evaporated, the block is imbedded in the usual way. The blocks should be so oriented that the fibers lie on the bottom where they can easily be seen when the paraffin is solid. After hardening, the material is fastened to wooden blocks in the usual way.

Little need be said about the orientation of the blocks in the microtome. For longitudinal sections, the block is so placed that the fibers lie perpendicular to the direction of the cut. The knife edge should cut at an angle of about 15°, as sharply inclined as possible, and the blade should be thoroughly moistened with gelatine water, as has been previously mentioned. The sections, even when very thin, can be readily unrolled on the knife blade and they are so resistant to handling that they are not easily damaged.

With a well sharpened knife, we have prepared cross sections 2μ in thickness, though for all purposes sections 6μ thick are equally suitable, and are more easily cut and handled. Longitudinal sections were satisfactorily cut as thin as 4μ , though here again sections 6μ revealed just as much and are more easily made. In longitudinal sections thinner than 4μ the fibers are torn from the imbedding material during unrolling, though fragments still remain. It is especially important in cutting longitudinal sections to have the block so adjusted that the section is cut in the same plane as that occupied by the fibers, otherwise only disappointing fragments will result.

The further treatment of the sections is simple. They are cemented to slides with the usual protein-glycerin mixture, and after drying are treated with benzol or xylol to remove the paraffin and then with ether to dissolve the celloidin. The removal of the celloidin is not essential if the sections are to be used for demonstration purposes, but it is necessary if microchemical work is to be done on the sections. Permanent mounts can be satisfactorily prepared with glycerine or glycerine-gelatine.

The method here described has been applied only to cotton fibers, where it has given excellent results. We are convinced, however, from our experience with it, that the method can be used to good advantage in the study of other fibers where extremely thin cross and longitudinal sections are required.

While the results of the investigations now in progress on these sections

will be reported later, it may be of interest to indicate here the importance of thin sections for work on cell-wall structure. The wall of cotton fibers is known to be lamellate and the lamellae to be composed of spirally wound crystalline fibrillae. Little or nothing is known, however, of the relations of these lamellae to one another; whether for example, the stratification is the result of an alternation of "wasserarmer" and "wasserreicher" layers of cellulose, of the presence of contact surfaces between the lamellae, or of an alternation of different chemical substances. From microchemical studies of the cross sections of the fibers it is hoped to increase our information on these points.

In the bast fibers of flax the spiral fibrillae composing the various lamellae run in opposite directions, in successive layers. In hemp and ramie fibers, the spirals run in the same direction, but at different angles. In cotton it is difficult to make a determination of the direction of the spirals in the different lamellae, because of the flattened and twisted nature of the fiber. If the fibers are treated with swelling reagents the spirals can clearly be seen, but it is not possible to distinguish the course of the fibrils in the separate lamellae. By using thin longitudinal sections and treating them with swelling reagents under pressure, it is possible to separate the lamellae more or less from one another and to study them separately. The lamellae cannot be sufficiently separated when entire fibers are treated in the same way, and it is only through the use of thin longitudinal sections that the matter can be successfully worked out.

The use of thin cross and longitudinal sections in X-ray studies will widen the possibilities of this most interesting method of studying cell-wall structure.

The breeding of cotton for new and better varieties can be materially aided by good cross sections of the fibers. Much can be learned about the character of the fiber from cross sections that is impossible to discover from observations of the fiber surface. Consequently, through a study of good cross sections, fibers with the most desirable qualities can be selected for breeding purposes.

It is hoped that the method here described will be of service in solving some of the problems in these fields of investigation.

MEDULLARY BUNDLES IN *LOBELIA PUBERULA*

ROLAND HOLROYD

(Received for publication March 17, 1928)

Accessory vascular bundles may be found in the cortex of certain plants in the Melastomaceae, Calycanthaceae, Cactaceae, etc.; in the pith in members of the Piperaceae, Cucurbitaceae, etc., or even in the medullary rays, as in the transmedullary bundles of *Citrullus vulgaris*, *Lagenaria vulgaris*, and other cucurbits previously described by the writer (4). Accessory pith or medullary bundles may represent independent stem strands, as in certain Begoniaceae, Melastomaceae, etc., or leaf-traces which penetrate the stem as in Piperaceae, Cucurbitaceae, and *Lobelia puberula*. The last-named species has been the subject of recent investigation by the writer.

Westermaier (5) found a greater amount of translocated food in plants that live through the winter on reserves stored in tubers or rhizomes than in those which develop perennial woody shoots. Thus, in the former there is a greater demand upon the conducting system which may be met by the development of accessory vascular bundles, a feature seen in *Lobelia puberula*. Plants having numerous flowers densely crowded together may develop accessory bundles to carry the requisite nourishment to all the developing seeds, as in certain species of *Campanula*. DeBary (1) found that the presence of large amounts of chlorophyll in the cortex may demand the development of an auxiliary conducting system, generally occurring in the form of cortical bundles, extrafascicular bundles previously described for *Momordica* (4), and ecto- and endocyclic sieve-tube connections, as traced by Fischer (3). In *Campanula rapunculoides* medullary phloem and vascular bundles were described by Col (2) but the subsequent work of Ydrac (6) failed to find these in the Lobeliaceae.

Lobelia puberula Michx. is a perennial herb growing in sandy soil from New Jersey to Iowa and south to Florida and Texas. In New Jersey it not infrequently attains a height of from four feet to five feet six inches, and not from one to three feet, as stated in some manuals. Differing from *Lobelia cardinalis*, *L. inflata*, *L. siphilitica*, *L. erinus*, and others which are perennial by offsets, *L. puberula* is truly perennial, existing through the winter by food stored in its caudex. This fact is important when it is remembered that *L. puberula* alone of the above-mentioned species is provided with accessory bundles.

METHODS

The usual paraffin technique was employed in the preparation of meristematic tips and seedling junctions. In tracing the progress of bundle

development through the axis of more mature parts, however, it was necessary to cut sections either by hand or by sliding microtome. The difficulty in this method lay in keeping the enormous number of sections in serial order while staining and mounting. Obviously each section could not be handled separately. The following simple method was employed. As quickly as the sections were cut they were placed on a disk of filter paper in a petri dish. On the paper had been ruled with a lead pencil a series of squares a little larger than the sections, the square in the upper left-hand corner bearing a cross to indicate the beginning of the series. The paper had been previously stained in 50 percent alcoholic safranin. Thus, as soon as the sections touched the paper, staining commenced. The dehydration was completed by pouring successive strengths of alcohol on the petri dish, allowing it to run slowly under the paper and soak through. This did not disturb the sequence of the sections. The counter stain was applied the same way. Sections were then removed from the paper disks, cleared, and arranged in order on the slides. Large-size rectangular cover slips were adjusted and a drop of stiff balsam was placed on the slide at one edge of the cover slip. By applying mild heat, the balsam melted sufficiently to run under the cover slip but did not disturb the arrangement of the sections in any way. On cooling, the balsam was firm and the slides could be handled without difficulty.

THE ROOT

The primary root is for the most part evanescent. Its vascular system, at first triarch, later splits lengthwise into a tetrarch and hexarch system toward the hypocotyl. The endodermis is a conspicuous feature and the adjacent pericambium or pericycle produces side roots in a $\frac{1}{3}$ spiral system. There is no pith and consequently there are no medullary bundles.

THE SEEDLING STEM

In seedlings seven to ten days old the basal part of the hypocotyl reveals an undifferentiated pith devoid of medullary bundles. Slightly higher a single patch of embryonic medullary cells with conspicuous nuclei appears. These cells start division and constitute the first rudiment of a medullary bundle which occupies the center or a position near the center of the pith. This bundle consists largely of phloem with a small amount of attendant xylem. From this bundle and from other medullary bundles subsequently formed, diverticula may pass into the adventitious side-roots. Sections from a somewhat higher part of the hypocotyl show that this medullary bundle divides into two more or less unequal bundles. By division of the larger one, three distinct pith bundles are formed. By a series of similar divisions, four, five, six, eight, or more medullary bundles arise. This process continues until, in late August and September, there may be from twenty to forty distinct medullary or pith bundles which have become irregularly distributed throughout the now greatly enlarged pith.

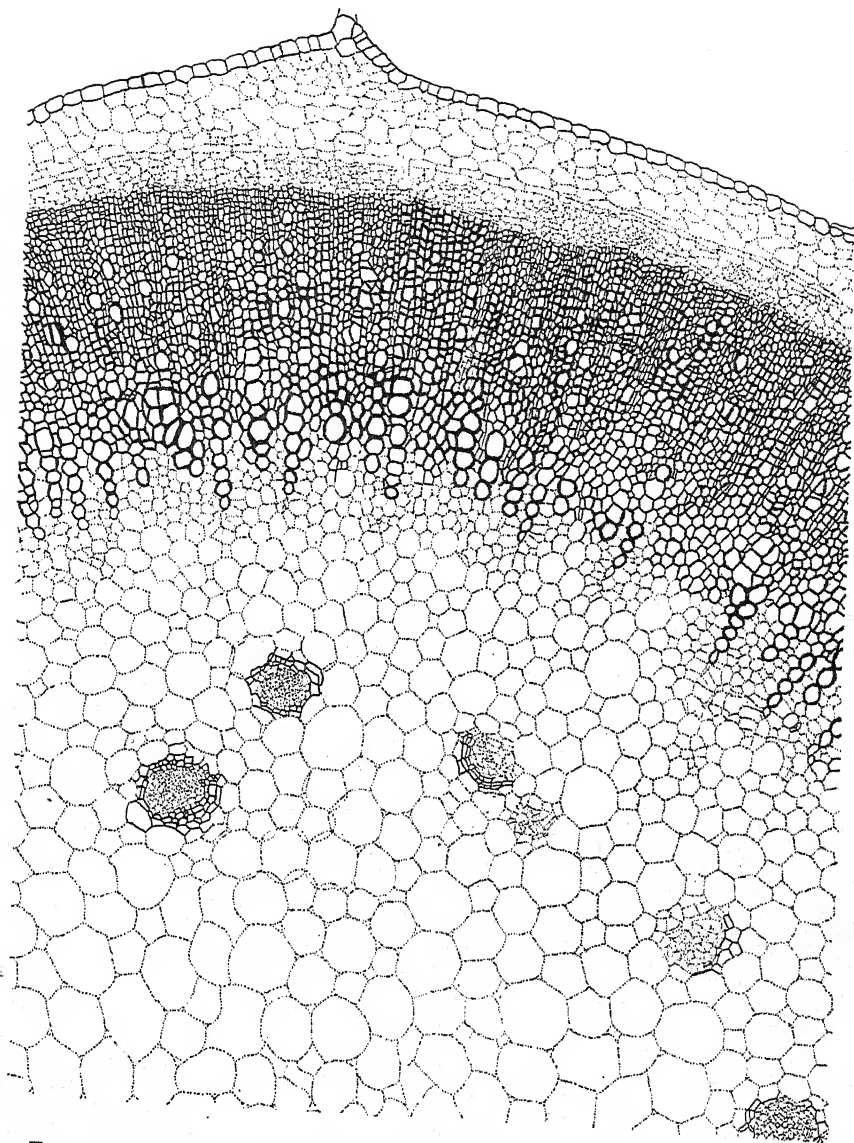
THE MATURE STEM

The stem shows typical dicotyledonous structure with one significant exception, namely, that no bast fibers are developed (text fig. 1). The absence of strengthening phloem can be readily accounted for by the fact that the medullary bundles furnish additional mechanical support.

The medullary bundles originate at the stem apex from four procambial strands (Pl. XXIX, fig. 1). At first only phloem is differentiated, probably because the conduction of elaborated sap downward to the caudex is of primary importance, and the normal phloem is present in not over-abundant amount. Both the normal and the medullary phloem are made up of typical elements. A periphloic cambium later develops which cuts off metaxylem, composed largely of pitted vessels, on its external surface. Thus there is no medullary protoxylem. This periphloic cambium develops first as a marginal line on but one side of the original medullary phloem. Later it completely encircles the phloem. The medullary metaxylem, at first often a restricted patch, in most cases becomes an encircling ring, thus constituting the whole an obconcentric medullary bundle (Pl. XXIX, fig. 2). These mature medullary bundles not infrequently show branching and in the lower part of the mature stems there may be as many as forty present.

Toward the caudex region the xylem is lost, at least on one side, and thus the medullary bundles become obcollateral and obbicollateral instead of obconcentric. This is evidently of importance in allowing the elaborated sap to flow out from the medullary phloem into the storing pith parenchyma. The medullary bundles do not persist into the very base of the caudex but lose their identity in the maze of side roots which issue from the base of the stem.

The medullary bundles in their course through the stem branch and re-branch in almost every internode. Not infrequently are two separate strands observed to rejoin after being distinct for a considerable distance within an internode. An example of this was seen in a plant about three feet high whose lowest internode showed seventeen distinct medullary bundles. At the node immediately above, two passed out into the lowest leaf. The second lowest internode thus showed but fifteen medullary bundles. However, in the fourth internode above the root the number seventeen was once more restored by the branching of certain of the remaining bundles. At the fifth internode three passed out through the leaf gap, but by branching of the remaining fourteen the number was brought up to thirteen in the sixth internode. Thus, throughout the length of the lowest internode of the stem, the number of medullary bundles varied from ten to seventeen, nor did there appear to be any regular diminution and subsequent restoration of the number from internode to internode. Above the sixteenth internode there was a marked lowering of the number until in the twenty-second internode, immediately below the flower cluster, they had entirely disappeared.



TEXT FIG. 1. Cross section through the upper median region of the stem below the flowering axis showing medullary bundles of the obcollateral and obconcentric types. The absence of bast fibers and the well marked endodermis are conspicuous features. $\times 180$.

THE LEAF

In mature flowering plants of *Lobelia puberula*, the medullary bundles rarely pass very far into the axis of inflorescence. A few enter the lower bracts. They chiefly leave the mature stem in pairs, sometimes in sets of three, four, or occasionally six and seven, as leaf traces. They pass through leaf gaps and become lateral appendages, withdrawing elaborated sap from the lower third of the leaf. They can be readily seen in the winged petioles. Beyond the petiole they pursue a course parallel to the main leaf-trace bundle derived from the normal bundle ring. This main bundle is almost normal in structure except that the phloem almost surrounds the xylem.

SUMMARY AND CONCLUSIONS

These results suggest the following conclusions:

(1) The presence of medullary bundles in the stem is probably a diagnostic character for *Lobelia puberula* Michx.

(2) The medullary bundles in this species constitute an auxiliary conducting system primarily for elaborated sap.

(3) They are developed from the primary meristem (Cf. Cucurbitaceae) in connection with the habit of the plant of perennially carrying food to the caudex.

(4) The bundles consist chiefly of typical phloem tissue whose evident function it is to carry downward elaborated food to nourish the rapidly enlarging caudex and also to supply the root.

(5) The bundles have their origin in the pith of the seedling hypocotyl in its lower median region where a single group of embryonic cells with conspicuous nuclei start proliferation.

(6) The bundles, at first composed of phloem only, later develop a periphloic cambium which, by developing metaxylem on its external face, makes the bundle collateral or obcollateral, then obbicollateral, and finally obconcentric.

(7) In the caudex the bundles become collateral or obcollateral, facilitating the escape of the elaborated sap into the pith tissue for storage.

(8) Accessory leaf-trace bundles pass out into the winged petioles where they drain elaborated sap from approximately the basal one-third of the leaf.

(9) Because these bundles are not extended into the axis of inflorescence much, if at all, beyond the lowest bracts but rather into the leaves, the writer concludes that they are not associated with the nourishment of flowers and fruits as in Cucurbitaceae but rather with the removal of elaborated sap from the general vegetative system, especially the larger median and upper leaves.

(10) While the considerable amount of lignified xylem associated with the large amount of phloem in the accessory bundles may aid in the passage upward of crude sap supplies, the likelihood seems to be that its main function is mechanical and strengthening.

The writer wishes to acknowledge his indebtedness to Dr. John M. Macfarlane for the helpful criticisms he has offered during the progress of this investigation.

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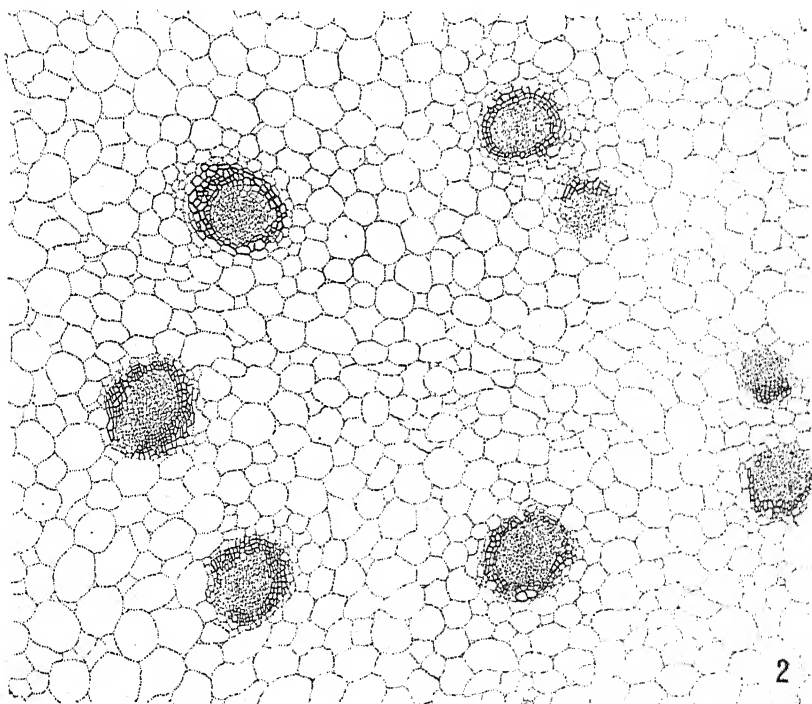
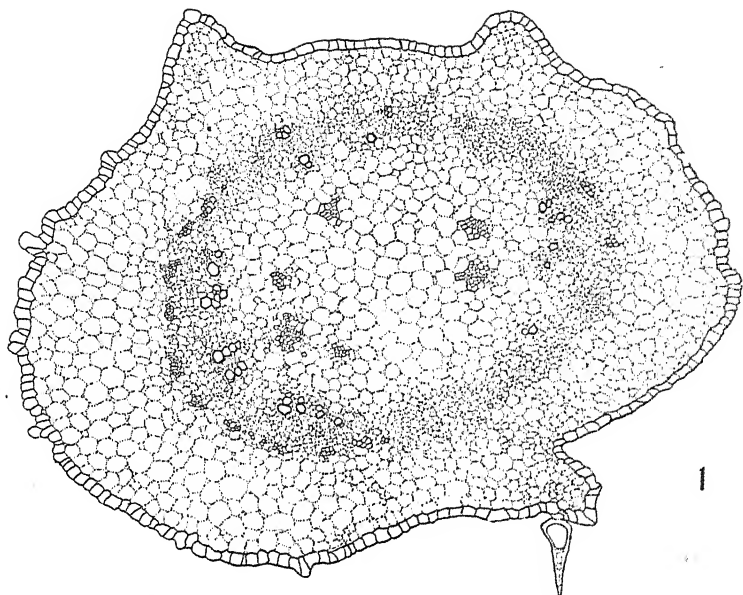
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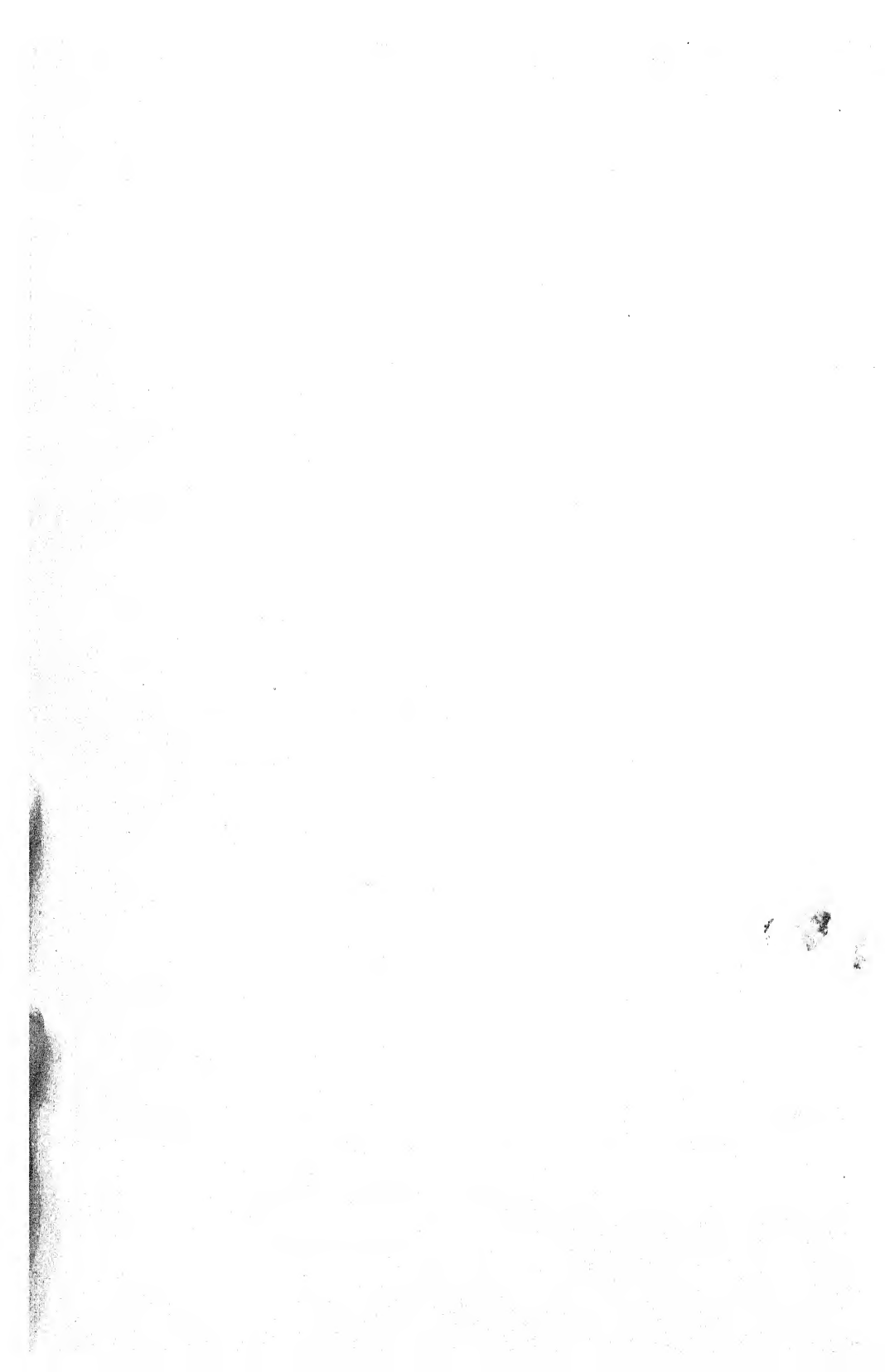
EXPLANATION OF PLATE XXIX

FIG. 1. Cross section of a seedling meristem showing the progressive differentiation of vascular tissue and the origin of medullary bundles as procambial strands. The original four have here split into six medullary strands. $\times 150$.

FIG. 2. Cross section of the pith in the lower median region of a mature stem. The larger bundles are here seen to be obconcentric with the periphloic cambium conspicuous between the phloem and the xylem. The smaller bundles are still collateral. $\times 180$.



HOLROYD: MEDULLARY BUNDLES



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SEASONAL VARIATIONS IN THE PHYSICAL AND CHEMICAL PROPERTIES OF THE LEAVES OF THE PITCH PINE, WITH ESPECIAL REFERENCE TO COLD RESISTANCE¹

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INTRODUCTION

In order to reach an understanding of the growth responses of a species, or of its ability to survive under the set of environmental conditions representing a given habitat, consistent studies of the seasonal variations in the physiology of that species are necessary, as well as consistent and parallel studies of the seasonal variations in the habitat factors. It is particularly important that the critical periods in the water and temperature relations of the species be recognized. These critical periods are not necessarily those of extreme drought or extreme cold. They are the periods when the plant is not adequately adjusted to the demands or effects of the environment. Evergreen leaves which can endure severe cold spells during the winter may be damaged or killed by late spring or early autumn frosts. The difference in the behavior of the leaves is accounted for by the differences in their physiological reactions at the different seasons. Similar critical periods may occur in the water relations of a plant. A warm period, especially if accompanied by wind, may often desiccate plant organs in the winter or early spring, which would not suffer from such climatic conditions if they occurred in the summer.

Knowledge of the critical periods in the water or temperature relations of a plant is necessary if our understanding of the climatic limits to its distribution is to be placed upon a sound physiological foundation. Throughout the greater part of the natural range of a plant it is physiologically so adjusted to the seasonal changes in its environment that only an unusual combination of conditions will have a destructive or detrimental effect upon

¹ Papers from the Department of Botany, the Ohio State University, No. 206.

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it or any of its organs. The closer a species approaches to the edge of its climatic range, the more likely it is to suffer destruction or damage in some or all of its parts at certain seasons, and the more likely it is to show unusual growth responses. Critical periods in the relation of a plant to its environment may occur only at intervals measured in terms of years, but usually this will not materially diminish their effectiveness in determining plant distribution, unless the span of time intervening between such periods is an extremely long one.

This paper reports the results of an investigation upon the seasonal variations in the physical and chemical properties of the leaves of the pitch pine (*Pinus rigida* Mill.). The properties studied were the water content of the leaves, the volume of sap expressed from the leaves under standard treatments and pressures, the sugar content of the leaves, and the osmotic value of the expressed leaf saps. The investigation was begun in October, 1925, and lasted over a period of nearly two years. The data to be presented are concerned with the water relations of the pitch pine, and the problem of cold resistance² in the leaves of this species.

Throughout its range the leaves of the pitch pine are subjected to periods of freezing weather during the winter months. In Ohio these periods are sometimes prolonged, and occasionally severe. The evergreen habit of the leaves of this species requires that the leaf cells be so adjusted physiologically during the winter months that they will not be destroyed by low temperatures. It will become clear in the following discussion that the pitch pine leaves are not resistant to freezing temperatures during the summer. This means that they must undergo a "hardening" process during the autumn months, and a corresponding "dehardening" process during the spring months. Although this paper reports data only for the pitch pine, similar results were obtained in investigations of the seasonal variations in the physical and chemical properties of the leaves of *Pinus laricio*, *Kalmia latifolia*, and *Rhododendron maximum*, indicating that the seasonal phenomena described for the leaves of this species are probably of general occurrence in the leaves of evergreens.

REVIEW OF LITERATURE

Dixon and Atkins (1) determined the seasonal variations in the osmotic value of the expressed leaf saps of *Syringa vulgaris*, *Ilex aquifolium*, and *Hedera helix*. The leaf samples were frozen in liquid air before the sap was expressed. The maximum osmotic value in the lilac was found to occur in August. In both of the evergreen species the winter osmotic values were higher than the values obtained in the summer. The seasonal

² The term "cold resistance" as used in this paper refers only to the ability of plant tissues to survive unharmed periods of freezing temperatures which ordinarily would be expected to cause damage or destruction of the tissues due to the formation of ice crystals within them.

range in the holly was from 18.91 atmospheres in March to 12.68 atmospheres in August; in the ivy it was from 17.66 (March) to 11.58 (August) for the leaves with a northern exposure, and from 18.77 (January) to 12.78 (August) for leaves with a southern exposure. These investigators concluded that "variations in the osmotic pressure are due to a large extent to fluctuations in the carbohydrate content of the cells, and to a smaller degree to changes in the concentration of the electrolytes."

Lewis and Tuttle (8) determined the variations in the osmotic value and sugar content of saps expressed from the leaves of *Picea canadensis*, *Linnaea borealis*, and *Pyrola rotundifolia* during the autumn, winter, and spring months. The maximum osmotic value was found toward the end of March in *Picea* and *Linnaea*, and apparently in December in *Pyrola*. In all three of these plants the osmotic value of the expressed leaf saps showed a considerable decrease in the spring. Variations in the sugar content of the expressed saps were found to be correlated very closely with variations in the osmotic value, and it was concluded that non-electrolytes were principally responsible for the variations in osmotic value. In a later paper (9) the same workers reported a rapid drop in the osmotic value of the expressed sap of the leaves of *Picea* from 20 atmospheres to 16 or 17 atmospheres during late April or early May as the leaf cells passed from the winter into the summer condition.

Gail (3) determined the seasonal variations in the osmotic value of the expressed leaf saps of certain western evergreens, both of the broad-leaved and of the needle-leaved species. His investigations extended over a period of three years. Leaves were ground to a pulp, usually frozen, and the sap expressed from a definite weight of the pulp under a definite pressure. This investigator found that the lowest osmotic pressure in the plants studied occurred in late June or in early July. The osmotic pressure gradually increased throughout the late summer and fall. Usually an abrupt increase occurred in December or January. From this maximum the osmotic pressure tended to decrease until June or July. If the temperature dropped low enough too suddenly in December or January, the usual rise in osmotic pressure did not occur. This was attributed to the fact that the sudden drop in temperature did not give the enzymes sufficient time to convert the starch into oils or soluble carbohydrates. Gail believed that the occurrence of a sudden fall in temperature before the winter increase in the osmotic pressure of the cell sap occurred might account for the winter-killing of pines and other evergreens.

Korstian (7), working on the osmotic value of the expressed leaf saps of trees, shrubs, and herbs in the Wasatch Mountains of Utah and vicinity, reported that most conifers showed a lower osmotic pressure in winter than in the summer or autumn, while the closely associated evergreen shrubs exhibited their highest osmotic pressures in the winter. He believed that this difference was due to the difference in the nature of the winter

food reserves in these two types of plants. Iodine-potassium-iodid tests showed starch to be lacking in both the conifers and evergreen shrubs in winter, although in the spring strong reactions were obtained. Osmic acid tests demonstrated the presence of large amounts of oils and fats in the coniferous leaves during the winter, but only traces in the evergreen shrubs. Korstian states: "These tests led to the conclusion that with the advent of cold weather in the autumn and early winter a large part of the starch in the conifers is converted into oil or fatty substances which are osmotically inactive and form emulsions having low osmotic concentrations. In the evergreen shrubs which showed little or no oil present the starch was evidently converted into soluble sugars, thereby materially increasing the osmotic concentration of the cell sap." It is difficult to see, however, how a decrease in the osmotic pressure of conifer leaves during the winter can be explained on the basis of a conversion of starch to oils, since both of these substances are osmotically inactive. Only if soluble organic substances were changed to oils would the osmotic pressure of the cell sap be affected. Certain discrepancies between Korstian's results and those of Gail will receive more attention later in this paper.

Goldsmith and Smith (4) have recently published the results of an extensive investigation upon the seasonal variations in the physico-chemical properties of the leaves and leaf saps of the Engelmann spruce (*Picea Engelmannii*). Leaves were collected from trees growing at altitudes of 7,200, 8,800, 11,300, and 11,500 feet in the Rocky Mountains near Colorado Springs. They were exposed to chloroform vapor for 36 hours in order to render the cell membranes permeable before expressing the sap. The water and carbohydrate contents of the leaves were determined, as well as the density, refractive index, osmotic value, electrical conductivity, hydrogen-ion concentration, and buffer action of the expressed sap. The water content of the leaves was found to be relatively high in the late spring, falling rapidly to a summer level, and slowly diminishing in the winter. The osmotic value of the expressed sap was lower in the summer than in the winter, a maximum occurring for samples from most altitudes in April or May. The carbohydrate content of the leaves showed a minimum in the summer, rising to a higher value in the winter. The general seasonal trend was usually found to be the same in all the properties studied regardless of altitude, but the values for any one date frequently showed a considerable variation depending upon the station at which the sample had been collected.

Seasonal variations in the amount and chemical nature of food reserves in the cells of evergreen leaves are in themselves of physiological significance, and undoubtedly are important factors in determining changes in the osmotic pressures and other properties of the cells from one season to another. Although the investigations of Schultz (19), Lidforss (10), Miyake (13), Tuttle (22, 23), and others have contributed important data to this subject,

our actual information is still fragmentary, and a general re-working and extension of investigations in this field is desirable. The methods used should also be subjected to a critical scrutiny. Meyer (11), for example, has shown that lack of regard for the limitations of the methods used has led to the identification of a substance commonly occurring in evergreen leaves as a fat, which in reality is not a fat at all.

METHODS

The leaf material used in these investigations was collected from pitch pines growing near the village of Sugar Grove, about 40 miles southeast of Columbus, Ohio. The trees from which leaves were gathered grew on the south-facing slope of a high ridge just above a sandstone escarpment about 100 feet in height. Several trees were used as a source of material, but all were about 25 years of age and all of them were subject to practically identical environmental conditions.

The leaves were placed, as rapidly as picked, into large test tubes, one and one-quarter inches in diameter and eight inches long. Each leaf sample was weighed, tubes of this size accommodating from fifty to sixty grams of leaves. If the leaf sample was to be frozen, the tube was plunged at once into a freezing mixture of ice and salt at a temperature of approximately -15° to -20° C. The samples were left in this freezing bath over night, the freezing mixture being renewed if necessary. Certain series of samples were frozen with solid carbon dioxide. These samples were allowed to remain in the collecting tubes, which were kept in ice water, until the hour and a half elapsed which was required to bring them from the field into the laboratory. Here they were immediately transferred, a small portion at a time, to a pint "Thermos" food jar. Each portion was thoroughly sprayed with solid carbon dioxide as soon as it was placed in the jar from a cylinder containing compressed gas and liquid. The mass of leaves and solid carbon dioxide was then tightly stoppered in the insulated food jar and set in a refrigerator over night. Leaves treated in this way are still cold when the jars are unstoppered the following morning. Tubes of leaves which were not to receive either of these freezing treatments were placed upon collection in ice water, transferred to a refrigerator as soon as the laboratory was reached, and kept there overnight. All leaf samples were collected between three and four o'clock in the afternoon, and determinations were made upon every set during the forenoon of the following day.

Saps were expressed from the leaf samples in a press of special design, of the cylinder and piston type, which has previously been found to give excellent service in the expression of saps from the leaves of a variety of species. This press has been described in detail in a previous paper (12). A materials-testing machine³ was used as a source of pressure. The

³ Placed at the disposal of the writer by the courtesy of the Department of Mechanical Engineering, the Ohio State University.

pressures used on each sample were, successively, one, two, three, four, and five thousand pounds per square inch. After each pressure had been attained the press was allowed to drain for two minutes before the next increment in pressure was applied. The volume of sap expressed under each pressure was measured. As soon as the expression of the sap was completed the graduated test tubes in which it was collected were tightly stoppered and placed in ice water pending determinations of the depression of the freezing point.

The methods of Gortner and Harris (6) were followed in the main in determining the depression of the freezing point of the expressed leaf fluids. Certain modifications in technique which expedite the determinations and avoid certain vexatious technical difficulties have been described by the writer (12).

The water content of the leaves was determined by desiccating approximately thirty-gram leaf samples to constant weight in an oven at 103° C.

Methods of sugar analysis were adopted only after preliminary experimentation indicated that they give satisfactory results with pitch pine leaves. Fifty to sixty grams of leaves were picked into large test tubes, weighed, and transferred at once into 500-cc. flasks, containing 400 cc. of aldehyd-free alcohol to which had been added one-half gram of calcium carbonate. Sufficient distilled water was added to the flask to adjust the alcohol to an eighty-percent concentration, allowance being made for the known water content of the leaves. The flasks containing the leaf samples immersed in alcohol were heated to the boiling point, and allowed to boil for five minutes to destroy the leaf enzymes. This heating of the flask was performed in the field, a small alcohol stove being used to heat the water bath. The flasks were then tightly stoppered, and upon being brought into the laboratory were stored in a dark place until used for the sugar determinations. Usually about one month elapsed between the collection of the samples and the sugar determinations.

Extraction of the sugars from the sample was completed in Soxhlet extractors. The alcoholic solution was drained from the leaf samples. The leaves were dried in an oven at 70° C., and crushed to a coarse powder. The powdered leaves were extracted for ten hours, using the alcoholic filtrate as the percolate. After extraction the volume of the alcoholic solution was made up to 500 cc. with 80-percent alcohol.

100 cc. of this alcoholic sugar solution was concentrated slowly on a steam bath, small amounts of distilled water being added from time to time to prevent too severe a contraction in the volume of the solution, until all traces of the alcohol had disappeared. The water solution of sugars was cleared with a minimum volume of a saturated solution of neutral lead acetate. One cubic centimeter was the amount generally used in these determinations. The precipitate was centrifuged from the solution, the residue being washed with water and re-centrifuged.

Potassium oxalate was used to de-lead the clarified solution. This was added at the proportion of .75 gram of potassium oxalate to each cubic centimeter of saturated neutral lead acetate solution used. The solution was finally filtered and made up to a volume of 250 cc.

Reducing sugars were determined by the cuprous titration method introduced by Shaffer and Hartmann (20), using the modified micro-tartrate-carbonate copper reagent described by Somogyi (21). The amounts of reducing sugars in the samples were read from a graph prepared by determining the volume of .005 *N* sodium thiosulfate solution required for the titration of a series of pure dextrose solutions ranging in sugar content from .2 to 2 milligrams. The amount of sugar in the total leaf samples was calculated from this value.

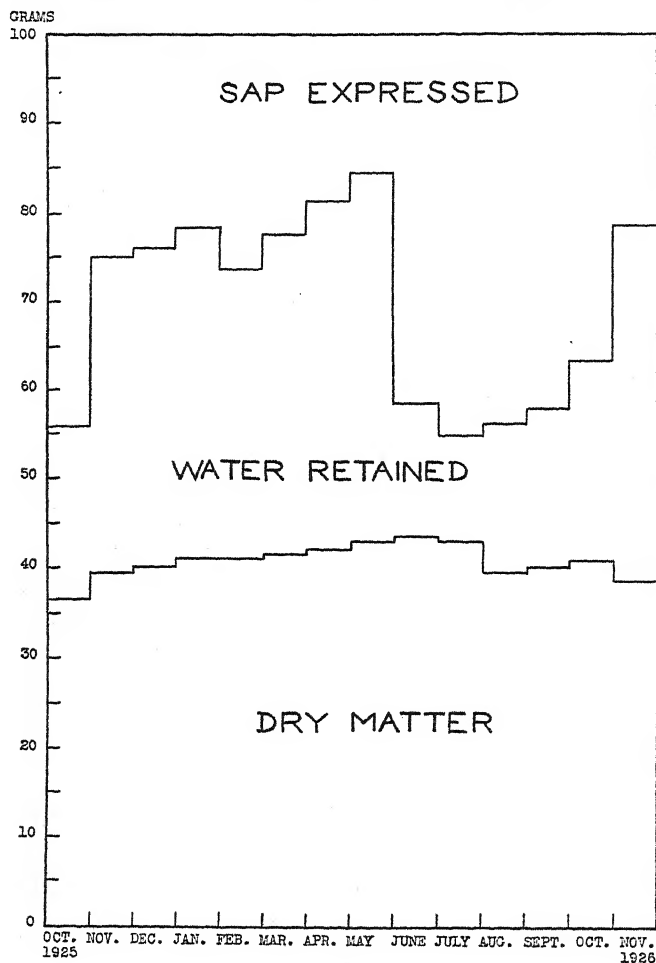
Inversion of the non-reducing sugars preparatory to their determination was carried out by the standard Herzfeld method. A 50-cc. sample of the water extract of sugars was used. After inversion the flask containing the sugar solution was cooled under the tap, and normal sodium hydroxid was added until the solution was distinctly alkaline. The final water extract of the pitch pine leaves contains a natural indicator which changes color at approximately the neutral point. This extract is a pale yellow in color when acid, and a deep orange red when alkaline. The volume of the neutralized solution was made up to 200 cc., and the determination of reducing sugars carried out on samples of one or two cubic centimeters, run in quadruplicate. The reducing sugars corresponding to the non-reducing sugars present were then calculated.

SEASONAL VARIATIONS IN THE WATER RELATIONS OF PITCH PINE LEAVES

Text figure 1 is a graphical presentation of the seasonal variations in the dry-matter content, the inexpressible water, and the water expressed as sap after freezing in an ice-salt bath for the 1925 leaves of the pitch pine. This series of determinations was begun in October, 1925. Text figure 2 presents graphically the results of a similar but more extensive series of investigations upon the seasonal variations in the physical properties of the 1926 leaves of the pitch pine, beginning in August, 1926. The successive areas of this figure represent, starting at the top, (A) the volume of sap expressed from unfrozen leaves, (B) the additional sap expressed from frozen leaf samples, (C) the additional sap expressed from samples frozen in solid carbon dioxide, (D) the inexpressible water, and (E) the dry matter content of the leaves. A standard pressure of 5,000 pounds per square inch was used in every determination. In both figures the vertical columns represent 100-gram leaf samples, so the figures are on a weight-percentage basis. In plotting these figures, the expressed sap is assumed to have the same density as water. This, although not strictly accurate, introduces no serious error for the present discussion, since the solution volumes of the solutes present in these saps, in their usual concentrations, are so small as

to be almost negligible. The error is in the direction of a slight increase in the volume of the sap.

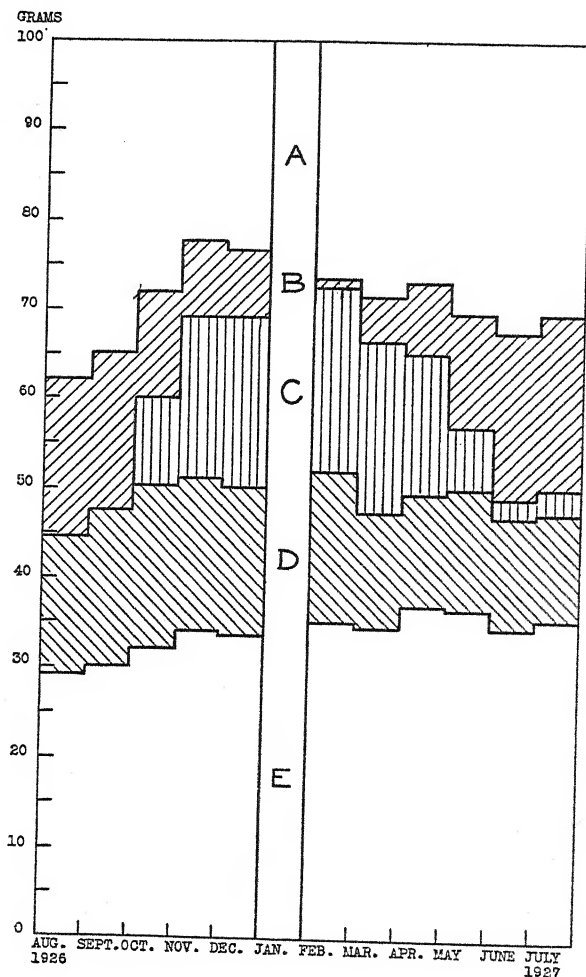
The highest leaf water contents were recorded in the recently developed leaves during the summer of 1926. The total water content of both the 1925 and 1926 leaves of the pitch pine shows a slow and mostly consistent



TEXT FIG. 1. Seasonal variations in the dry-matter content, inexpressible water, and water expressed as sap after freezing in an ice-salt bath for the 1925 leaves of the pitch pine. Pressure, 5,000 pounds per square inch.

diminution during the autumn and winter months. The minimum water content was reached in the late spring in both the 1925 and 1926 leaves. A relatively high transpiration rate while absorption is still retarded by a cold soil possibly explains the occurrence of the minimum leaf water content at this season. This suggests that late spring may sometimes be a critical

period in the water relations of the leaves of the pitch pine. After the spring minimum there is a slight increase in the water content of the leaves during the following summer months. The 1926 leaves during the year 1926-27 consistently show a water content from four to nine percent



TEXT FIG. 2. Seasonal variations in the dry-matter content, inexpressible water, and water expressed as sap after different treatments for the 1926 leaves of the pitch pine. *A*, volume of sap expressed from unfrozen leaves; *B*, additional sap expressed from leaf samples frozen in an ice-salt bath; *C*, additional sap expressed from samples frozen in solid carbon dioxide; *D*, inexpressible water; *E*, dry matter content. Pressure, 5,000 pounds per square inch.

higher than the 1925 leaves showed for the corresponding months during the year 1925-26. The extreme seasonal range for the water content of the matured pitch pine leaves during the first year of their existence did not exceed eight percent for either the 1925 or 1926 crop of leaves.

A number of investigators have ascribed to a high dry-matter content of plant organs during the winter months an important rôle in preventing the freezing to death of those organs. The corresponding low moisture content of the tissues means a higher concentration of solutes in the vacuoles, and a larger proportion of water held by imbibitional forces in the leaf cells. The greater proportion of the cell solutes and the larger proportion of water bound by molecular forces make it more difficult to abstract water from the cells during the freezing process. Reduction in the moisture content of the tissues is a negligible factor in the winter hardiness of pitch pine leaves. In these two series of determinations the moisture content was, at its minimum during the months when freezing weather may prevail, only two percent less than the maximum water content of the leaves the following summer, during which period, as will be shown in the following discussion, they were readily killed by freezing. The minimum leaf water content occurs in the late spring, at a season when the leaves of this species are seldom subjected to freezing temperatures.

Both figures show the marked seasonal variations which occur in the volumes of water which could be expressed as sap after freezing the leaves in an ice-salt bath. The amount which could be expressed for the 1925 leaves shows a sharp decrease in the autumn. A similar but more gradual decrease is shown in the autumn for the 1926 leaves. During the months November 1925 to May 1926, inclusive, relatively small amounts of sap could be expressed from the 1925 leaves. During the corresponding months in 1926-27 the same is true in general for the 1926 leaves, except that in October and May intermediate amounts of sap could be expressed. Between May and June the amounts of sap which could be expressed from the leaves increased markedly. In the 1925 leaves this increase occurred in one jump, in the 1926 leaves it occurred during the period April to June. During the summer months relatively large amounts of sap could be expressed from the leaves of both series. In the 1925 series the volume of expressible sap is again shown to diminish markedly between October and November.

The cause of this reversible seasonal variation in the volumes of sap which can be expressed from the leaves of the pitch pine after freezing at the temperature of an ice-salt bath does not lie in the seasonal variation in the water content of the leaves. Relative to the enormous variation in the amounts of sap which can be expressed, the small seasonal variation in the water content of the leaves is totally inadequate as an explanation of the phenomenon. Neither can anatomical changes in the leaves which might cause a variation in the resistance offered by the leaves to pressure provide an explanation. The fact that this seasonal variation is reversible completely precludes its explanation on the basis of any permanent anatomical change. The cause of this phenomenon must be sought in changes in the reversible chemical and physical equilibria existing in the cells of the leaf tissue.

Newton (14, 15) has described a similar phenomenon in the leaves of winter wheat. He found that it was possible to express much larger volumes of sap from the leaves of winter hardy wheats during the early autumn or late spring months than during the winter months. In the winter, even prolonged exposure of the wheat leaves to a cryohydrate mixture of snow and calcium chlorid, with a theoretical freezing point of $-54.9^{\circ}\text{C}.$, failed to permit the expression of any appreciable amounts of sap. Newton believed that the volume of sap which could be expressed was determined by the dry-matter content, and by the physical state of the cell colloids. He believed it probable that during the winter months the moisture content of the leaves was largely in the form of a gel, and because of the tenacity with which the water was "bound" it was impossible to force any appreciable amounts of sap from the tissues under pressure. The following explanation of this similar phenomenon in the leaves of the pitch pine is essentially the same as that given by Newton for wheat. Seasonal variations in the dry-matter content, however, appear to be of negligible importance in determining the amount of water which can be expressed as sap from pitch pine leaves.

For the sake of lucidity in the following discussion the present status of our conception of the mechanics of the freezing process as it occurs in leaves will be briefly reviewed. In the freezing of leaves water usually crystallizes only in the intercellular spaces. The freezing of water begins in the films on the cell walls bounding the intercellular spaces because this water is relatively free from solutes as compared with the vacuolar sap. Water is withdrawn from the cell walls by the forces of crystallization operating in the intercellular spaces, and the equilibrium of the whole cell system is disturbed. The walls imbibe additional water from the protoplasm which in turn imbibes water from the cell sap. The imbibitional forces in the cell wall and the protoplasm as well as the osmotic pressure of the cell sap tend to prevent movement of water out of the cell. As the process proceeds it becomes increasingly difficult to abstract water from the cell and eventually a condition of equilibrium is reached. The lower the temperature, the greater the forces exerted in crystallization, and the more water will be taken from the cell. The loss of water from the cell, if severe enough, will result in the destruction of the protoplasmic organization, either through direct desiccation or by concentrating the cell sap to a point where the increased salt content or hydrogen-ion concentration will operate to coagulate some of the constituents of the protoplasm. It is important to note that it is the withdrawal of water from the interior of the cell which, directly or indirectly, causes disorganization of the protoplasm when ice forms in plant tissues.

The relatively small volume of sap which can be expressed from the leaves of the pitch pine during the winter months after freezing in an ice-salt bath indicates a physico-chemical organization of the cells at this

season which resists the formation of ice in the leaf tissues. The water-retaining capacity of the tissues has so greatly increased that it is effective in preventing or checking the withdrawal of water from the cells by the forces of crystallization operating in the intercellular spaces during the freezing process. Normal winter temperatures in central Ohio seldom go below 0° F., and then only for a day or two at a time. These temperatures are not sufficiently low to kill the leaves of the pitch pine under natural conditions because they do not engender forces of crystallization in the water films lining the intercellular spaces of sufficient magnitude to abstract any considerable amounts of water from the cells in their winter hardened condition.

The increased water-retaining capacity of the leaf tissues indicates that during the winter months the proportion of bound water has increased at the expense of the free water. Some of this bound water represents the water of hydration of solutes present in the cell sap, and with an increase in osmotic pressure a larger proportion of water is bound in this way. The amount of water of hydration is relatively small however, especially in leaves such as those of the pitch pine, in which, as will be shown later, the winter osmotic pressure is not high. The vast majority of the water is bound by the forces of absorption and imbibition, which are engendered principally by the hydrophilic colloids present in the leaf cells.

These considerations have led to the postulation that during the increasingly cool autumn months a gradual accumulation of hydrophilic colloids, principally in the gel state, takes place within the leaf cells. Gels bind relatively larger amounts of water than sols; hence it is believed that the accumulated colloids are principally in the gel state. Substances in the sol state tend to pass into gels at low temperatures, and it is probable that most of the gels arise in this way. Some may also be elaborated from non-colloidal materials within the cell. Such seasonal shifts in the hydrogen-ion concentration and of the salt and soluble carbohydrate content of the cell sap may also occur as will increase the water-binding capacity of the cell colloids. No evidence has yet been obtained regarding the chemical nature of the colloids involved.

During the late spring months, the marked increase in the volume of sap which can be expressed under pressure indicates that the proportion of bound water in the leaf tissues has undergone a pronounced diminution, and that the leaf cells are no longer resistant to freezing at the temperature of the ice-salt bath. The water-binding gels, perhaps under the influence of moderating temperatures, have apparently been broken down to sols or non-colloidal compounds. Therefore, during the summer months the imbibitional resistance offered by the cell colloids to the movement of water out of the cell during the freezing treatment is greatly reduced. The protoplasm is readily disorganized and the cells killed by the freezing treatment, with a resultant increase in the yield of sap.

While the seasonal variation in the total water content of the leaves of the pitch pine is relatively inconsiderable, the seasonal variation in the relative proportions of bound and unbound water is marked, and undoubtedly of prime significance in the cellular physiology of the leaves of this species. It is believed that the seasonal behavior of the cell colloids is the important factor involved in this phenomenon, and that the hardening and dehardening of pitch pine leaves are principally to be explained on this basis.

Rosa (17), in his studies on the hardening process in vegetable plants, arrived at conclusions similar in general to those expressed in this paper, but reached by a different experimental route. He found that the hardening process in certain vegetable plants was accompanied by a marked increase in the water-retaining power of the tissues, due chiefly to an increase in the imbibitional forces in the cell. Hardened plants contained larger amounts of bound water, as measured by the dilatometer, than unhardened plants. The pentosan content of the hardened plants was found to be greater than that of the unhardened plants, and this investigator believed that this may function as water-retaining material in the protoplasm.

Newton and Brown (16) found that the percentage of proteins and pentosans in the total dry substance of wheat plants does not increase in the fall during the hardening process, and that the marked rise in the concentration of these substances in the expressed juice of the plants is due to a reduction in the moisture content of the tissue. Proteins were found to constitute the bulk of the cell colloids, pentosans being restricted almost entirely to the structural parts of the plant. Even as cell-wall components it is possible that these pentosans play a rôle in cold resistance. As has been previously pointed out, large imbibitional forces in the cell wall will resist withdrawal of water during the freezing process, with the result that smaller demands will be made upon the cell contents for water.

Doyle and Clinch (2) determined the seasonal variations in the water-soluble pentosans, the pentosans subsequently extractable with one-percent hydrochloric acid, and the remaining pentosans, extractable only with twelve-percent hydrochloric acid, in the leaves of a number of coniferous species. They could discover no relations, seasonal or otherwise, between hardness and pentosan content in the leaves of the species studied.

Text figure 2 also shows that the volume of water which can be expressed as sap from unfrozen samples of leaves is, in general, greater in the summer than in the winter. This determination was added to the 1926 series primarily as a check upon the hypothesis that colloidal gels accumulate in the leaf cells in winter, resulting in an increase in the proportion of water bound by molecular forces. It seems probable that a greater resistance would be encountered in expressing sap from the living leaves in the hardened winter condition, provided a large proportion of the water was bound (due to the presence of colloidal gels), than would be met in expressing

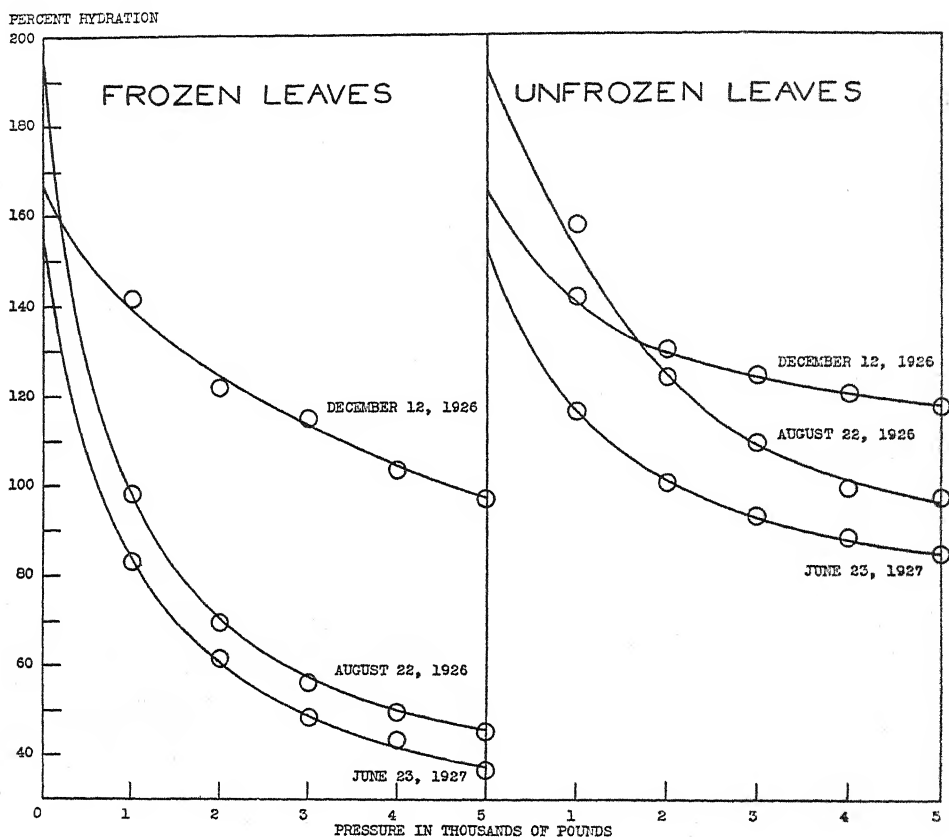
water from the leaves in the unhardened summer condition in which it is presumed that few colloidal gels are present in the cells, and that a relatively large proportion of the water is unbound. The results of this determination, although not in themselves to be considered substantially convincing, are nevertheless contributory evidence in support of the conception that gels accumulate in the leaf cells of the pitch pine during the winter. The high imbibitional forces exerted by these gels apparently resist the applied pressure during the winter months and reduce the volume of sap which can be expressed.

The volume of sap expressed from the leaves of the pitch pine after freezing in solid carbon dioxide, as shown in text figure 2, shows some minor fluctuations, but is essentially constant from one month to the next, regardless of season. The leaves of this species, even in the hardened winter condition, are not appreciably resistant to freezing at the temperature of solid carbon dioxide (about $-80^{\circ}\text{C}.$). When sprayed with the solid carbon dioxide the leaves freeze almost instantly, becoming hard and brittle. Freezing leaf samples in the winter condition with solid carbon dioxide results in winter yields comparable with those which can be obtained in the summer by merely freezing the samples in an ice-salt bath.

When figure 2 is interpreted from the standpoint of bound and unbound water, each of the areas represented has a significance for this conception. A certain proportion of the water present in the leaf tissues cannot be expressed, even after freezing them at the temperature of solid carbon dioxide and subjecting them to a pressure of 5,000 pounds per square inch. This probably represents roughly the water held in the cell walls (area *D*). Another fraction of the water present can be expressed from the living leaves at this pressure and this can probably be taken as an approximate measure of the free water (area *A*). A third fraction of the water cannot be expressed from unfrozen leaves, but can be expressed after freezing at the temperature of an ice-salt bath. This represents the water which, although not free, is not sufficiently bound by imbibitional forces to resist the forces of crystallization at a temperature of an ice-salt bath (area *B*). Water bearing this physical relation to the tissues is proportionately large in amount in the summer and proportionately small in the winter. Finally, there is the water fraction which cannot be expressed after the leaves are exposed to the temperature of an ice-salt bath, but which can be expressed after exposing them to the temperature of solid carbon dioxide (area *C*). This represents water bound in the tissues by forces sufficiently powerful to resist the forces of crystallization at a temperature of approximately -15° to $-20^{\circ}\text{C}.$ Water of this category is large in amount only during the winter months. This water is probably bound principally by the intracellular colloids and is the bound water of most importance from the standpoint of cold resistance.

This freezing and high-pressure treatment of leaves appears to furnish

a method of measuring the bound water within leaf tissues which may prove to be of considerable utility in physiological work. A thorough analysis of the distribution of water within the leaf tissues from this standpoint



TEXT FIG. 3. Pressure dehydration curves for pitch pine leaves.

requires determinations of the water content of the leaves, and of the volume of sap which can be expressed under a standard pressure from unfrozen leaf samples, from leaf samples frozen at the temperature of an ice-salt bath, and from leaf samples frozen at the temperature of solid carbon dioxide.

In text figure 3 are plotted pressure dehydration curves for leaves of the pitch pine, unfrozen and frozen in an ice-salt bath, as determined during the summer of 1926, the winter of 1926-27, and the summer of 1927. In these curves, the percent hydrations of the dry substance of the leaf under a series of successively increasing pressures are plotted as ordinates against the corresponding pressures as abscissae. These curves were not arbitrarily chosen for the purpose of this figure; other curves for the same

seasons are essentially similar. There is a progressive change in the shape of the curve from summer, during the autumn, to the winter, and the reverse change takes place during the spring.

A marked difference is shown in the pressure dehydration curves for the frozen pine leaves between winter and summer conditions. The difference is shown not only in the shape of the curve but in the magnitude of the observed values at different pressures. On August 22, 1926, the hydration of the leaves rapidly diminished with increase in pressure. That the leaf tissues offered little resistance to the expression of sap is shown by the fact that dehydration proceeds most rapidly at the lower pressures. By December 12, 1926, the leaves have passed into the winter hardened condition. Although the water content of the leaves dropped only 4 percent between August and December, the hydration of the leaves is much higher at each pressure in December than in August. The final hydrations are 99 percent in December and 47 percent in August. The shape of the curve shows clearly the greater resistance which is encountered in decreasing the hydration of the dry matter of the leaf in December. This shows that the pressure exerted upon the leaf sample is operating against a force not present in August—the water-binding capacity of the colloidal gels, which have accumulated during the autumn months, and which have not been destroyed by the freezing treatment. By June 23, 1927, the 1926 leaves have reverted to the unhardened condition, and the pressure dehydration curve for this date parallels closely the curve for August 22, 1926. The only important difference between the two curves is the larger decrease in the hydration of the tissues shown under the first increment of pressure on August 22, 1926, due to the larger water content on that date.

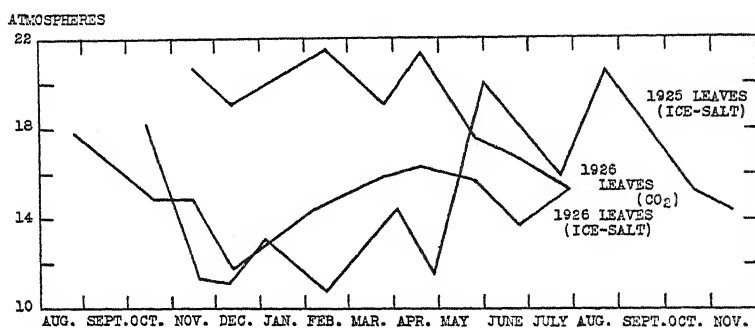
Pressure dehydration curves for unfrozen leaves of the pitch pine show a decided difference between the winter and summer condition of the leaves. The curves for August 22, 1926, and for June 23, 1927, are essentially similar, the difference in the magnitudes of the percentage hydration values being principally due to the higher water content of the leaves on August 22, 1926. The curve for the hardened leaves on December 12, 1926, shows clearly a greater resistance to dehydration on that date. An increase in the imbibitional forces in the leaf cells, contingent upon an increase in the amount or water retaining capacity of the cell colloids, seems to be the only feasible explanation. The reversibility of the phenomenon is in itself a convincing piece of evidence on behalf of this view.

SEASONAL VARIATIONS IN THE OSMOTIC VALUE OF THE EXPRESSED LEAF SAPS

Text figure 4 represents the seasonal variations in the osmotic value of the expressed leaf saps of the pitch pine for both the 1925 and 1926 series of determinations. Two curves have been drawn for the 1926 series. One

represents the osmotic value of the sap expressed after freezing the leaves in an ice-salt bath, the other the osmotic value of the sap expressed after freezing the leaves in solid carbon dioxide. Only the ice-salt freezing method was used on the 1925 leaves.

The osmotic value of the expressed leaf sap of a plant reflects the effect of numerous minor fluctuations in the environment and in the internal metabolism of the plant. It is, in fact, an index which is sensitive to any change in the environment which affects the water relations, synthetic processes, enzym activity, and respiration of the plant. In the present discussion, however, attention will be directed primarily to the major variations in this value which accompany changes in the plant's environment associated with the march of the seasons.



TEXT FIG. 4. Seasonal variations in the osmotic value of saps expressed from pitch pine leaves.

Sap expressed from both the 1925 and the 1926 pitch pine leaves after freezing in an ice-salt mixture shows a decrease in the osmotic value during the autumn months, and an increase in the late winter or spring. When the 1926 leaves were frozen in solid carbon dioxide, however, the contrary result was obtained; the osmotic value of the expressed sap was higher during the winter than in the summer, although the seasonal range does not appear to be great.

The explanation of this contradiction in results follows readily from the previous discussion. When the leaves are in their hardened winter condition an ice-salt bath does not provide a low enough temperature to cause any appreciable extraction of water from the leaf cells by the forces of crystallization operating in the intercellular spaces. The protoplasm is not destroyed, the amount of sap expressed is small, and it contains a relatively small concentration of solutes, because the cytoplasm is impermeable to many of the dissolved substances in the vacuolar sap. The result is a low osmotic value for the expressed sap, which does not indicate the true conditions in the living cell.

In order to disorganize the protoplasm during the winter months a more

drastic treatment is necessary. Solid carbon dioxid provides a low enough temperature to accomplish this. With this treatment there is no differential expression of the sap from one season to the next, and physical measurements made upon the expressed sap of hardened leaves are comparable with those made upon the expressed sap of unhardened leaves. During the winter, at least, the osmotic value obtained for saps expressed after this treatment may be taken as much more reliable than that obtained after freezing the sample in ice-salt brine. It will be noted that in the 1926 leaves a wide divergence between the results obtained by these two methods is found only in the winter; during the summer the results of the two methods check more closely. The unhardened leaves in their summer condition are readily frozen in an ice-salt mixture, and representative samples of sap can be expressed.

As a further check upon summer and winter osmotic values of the sap expressed from pitch pine leaves, leaf samples were prepared by a grinding method. The leaves were thoroughly shredded through a meat grinder provided with a fine knife, the sap expressed, and the osmotic determination made immediately. In table I are presented representative data comparing the results obtained by this method with those obtained by freezing with solid carbon dioxid.

TABLE I

Date	Sap Expressed (cc.)		Osmotic Value (atmos.)	
	Frozen	Ground	Frozen	Ground
August 3, 1927.....	47.6	42.9	15.91	18.68
January 22, 1928.....	41.0	31.3	21.12	25.73

These comparative data show that, although the grinding method results in higher osmotic values than the freezing method, the two methods agree in indicating higher winter values than summer values. This corroboration of the results of the solid carbon dioxid freezing method demonstrates beyond question the unreliability of winter determinations of osmotic value after freezing in an ice-salt brine.

The grinding process apparently exerts a bruising and tearing action on the hardened leaf cells sufficiently severe to disorganize the protoplasmic complex with which the condition of hardness is associated. It is also noteworthy that the amounts of sap expressed after grinding are lower than the amounts expressed after freezing in solid carbon dioxid. The water-holding capacity of the cells is not as completely destroyed as by the freezing method. The fact that less of the water in the leaf is expressed as a component of the sap is probably a partial explanation of the higher values obtained with the grinding method. Oxidations or other chemical processes occurring during the grinding process may also tend to raise the

value. On the other hand, during the expression of sap from unground frozen leaves, it is possible that a larger proportion of the solutes is filtered out of the sap in passing through the unruptured tissues than when it is expressed from ground tissues. There is no exact method of ascertaining which of these methods comes closest to indicating the value of the vacuolar sap as it exists in the leaf cells but both methods are apparently sufficiently reliable when comparative data only are necessary.

From the standpoint of cold resistance, winter osmotic pressures are the significant ones. Results of this investigation show definitely that the osmotic value of the expressed leaf saps is slightly higher during the winter months than during the summer. It appears that the maximum osmotic value of the expressed leaf saps occurs in the early spring, followed by rapid diminution in this value during the spring months. Similar results have been obtained by other investigators. A high concentration of solutes in the cell sap tends to prevent a movement of water out of the plant cell during the freezing process, although this factor is decidedly secondary in importance to the imbibitional forces within the cell. The osmotic values of the expressed leaf saps of the pitch pine, which seldom exceed one atmosphere even in the coldest weather, apparently permit us to dismiss the osmotic pressure of the cell sap as a factor of minor importance in the cold resistance of the leaves of this species.

It must be remembered, however, that all of the water expressed as sap after freezing or grinding the leaves is not available as a solvent in the leaf cells. According to the conceptions advanced in this paper, a large proportion of the water in the cell is bound in the winter and is thus unavailable as a solvent. The osmotic pressure of the vacuolar solution may therefore be much greater than the value obtained by freezing-point determinations upon the expressed sap, and possibly of greater significance than the experimental data seem to show.

The discrepancies between the results of Gail (3) and those of Korstian (7) on seasonal variations in osmotic pressures may be due to differences in the treatment of the leaves before the expression of the sap. Gail found that in general the evergreens of Idaho, both needle-leaved and broad-leaved species, attained their maximum osmotic pressures in the winter and their minimum osmotic pressures in the summer. The maxima ranged from 31 to 41 atmospheres, which is considerably higher than the values obtained for the pitch pine studied in this investigation. Korstian reported, on the other hand, that conifers in general showed low osmotic pressures in winter as compared with summer values, while in evergreen shrubs the reverse was true. Both of these investigators worked upon the yellow pine (*Pinus ponderosa*) and Douglas fir (*Pseudotsuga mucronata*). Korstian found the January osmotic pressure in the yellow pine to be 9.0 atmospheres as compared with a July osmotic pressure ranging from 12.6 to 18.6. Gail reported the January value in this species to be 26 atmospheres, the

February value to be 31 atmospheres, and the July value to be 16.5 atmospheres. Korstian's winter values for Douglas fir are: January 4, 6.9 atmospheres, January 25, 11.1 atmospheres, February 28, 8.0 to 8.9 atmospheres. In July values ranging from 14.0 to 20.9 atmospheres were recorded, with the average closer to the higher value. Gail reported a January osmotic pressure of 29.5 atmospheres and a February osmotic pressure of 35 atmospheres for this species. In July an osmotic pressure of 20 atmospheres was recorded. All of the values taken from Gail's work are the averages of determinations made during two successive years.

It is improbable that climatic differences between northern Idaho and the Wasatch Valley region of Utah could cause a complete inversion in the seasonal osmotic behavior of the leaves of these two species from the one region to the other. Gail ground the leaves thoroughly before expressing the sap. Sometimes the resulting pulp was frozen, but he reported that this had very little effect on the freezing-point depression of the expressed sap. Korstian froze the leaf samples in an ice-salt bath. Judging from the results of the present investigation, the writer is inclined to ascribe the discrepancy in the results of these two investigators to the different treatments employed. Gail's results appear to be more reliable than those of Korstian. The grinding treatment was probably effective in disorganizing the protoplasm of the hardened leaf cells, but Korstian's freezing method was probably not sufficiently severe to accomplish this. The lower osmotic values recorded by him in certain evergreen trees during the winter months were probably simply due to the fact that a representative sample of sap could not be expressed from the hardened leaves. It is possible that Gail's results may run a little high as compared with a suitable freezing method. It has been pointed out previously that in the pitch pine, at least, a smaller proportion of the water present in the leaves can be expressed after grinding than after freezing in solid carbon dioxide, and this will have the effect of slightly elevating the osmotic value.

Both of these investigators made determinations for the evergreen shrubs *Ceanothus velutinus* and *Pachystima myrcinites*. Korstian's results were substantiated by Gail, who also found higher winter than summer osmotic values in the expressed saps from leaves of these two species. Korstian apparently succeeded in obtaining representative samples of sap from the leaves of the evergreen shrubs during the winter but not from the conifers. The reason for this is not entirely clear to the present writer. Several explanations appear possible, but lack of a close familiarity with the species, the conditions under which the samples were collected, and the exact experimental methods used, make it inadvisable to enter upon a discussion of this point here.

SEASONAL VARIATIONS IN THE SUGAR CONTENT OF PITCH PINE LEAVES

Table 2 summarizes the seasonal variations in the reducing, non-reducing, and total sugar content of the 1926 leaves of the pitch pine over the period from September, 1926 to July, 1927. All reducing substances remaining in the water extracts are assumed to be sugars, and are calculated as dextrose. The amount of sugar is expressed as a percentage of the fresh weight of the leaves. Moisture content data are included so that comparisons on the dry weight basis may be made.

TABLE 2. *Seasonal Variations in the Sugar Content of Pitch Pine Leaves*

Date 1926-1927	Moisture Content (percent)	Percent of the Fresh Weight of Leaves		
		Reducing Sugars (as Dextrose)	Non-reducing Sugars (as Dextrose)	Total Sugars (as Dextrose)
Oct. 28.....	63.0	2.20	.71	2.91
Nov. 13.....	61.8	2.23	1.55	3.78
Jan. 10.....	62.0	2.77	1.33	4.10
Apr. 6.....	60.6	2.05	1.15	3.20
May 1.....	58.2	1.81	2.34	4.15
June 23.....	60.6	1.49	1.16	2.65
July 27.....	59.4	1.58	1.00	2.58

This table is sufficiently self-explanatory to obviate the necessity for any detailed discussion but in general it is to be noted that the sugar content is greater during the winter months than during the other seasons of the year. The total sugar content apparently reaches a minimum during the late summer or early autumn, increases in late autumn, remains relatively high during the winter and early spring months, and decreases in the late spring. The autumnal accumulation of sugars probably results from temperature effects upon the equilibria between soluble and insoluble carbohydrates. At low temperatures starch and perhaps other complex insoluble polysaccharides tend to be split into the simpler soluble sugars. The disappearance of the soluble carbohydrates in the spring is probably due either to their reconversion to insoluble carbohydrates, to their rapid utilization in early spring growth, or perhaps to both processes. A detailed discussion of the processes and internal equilibria governing the seasonal variation in the sugar content of the leaves of the pitch pine is beyond the scope of this paper.

There are two known ways in which an increased sugar content of the leaves may be important in rendering them resistant to cold. The effect of sugars in increasing the osmotic pressure of the cell saps will, as has previously been pointed out, aid the cell in a minor way in resisting the withdrawal of water during the freezing process. The seasonal variations in the sugar content of the leaves parallel in general the seasonal variations as determined for the osmotic values of the expressed sap, both maxima

occurring in the winter. It is probable, as other workers (1, 8) have pointed out, that the seasonal variations in the soluble carbohydrate content of the sap are principally responsible for the seasonal variations in the osmotic value of the expressed sap. Sugars in solution in the cell sap also exert a "protective action" on the proteins within the cell, checking or preventing their coagulation due to any concentration of salts or changes in the hydrogen-ion concentration incident upon the withdrawal of water during the freezing process. This protective action of sugars against protein precipitation has been pointed out by Gorke (5) and Schaffnit (18). Although it would be difficult to determine the importance of this process in living cells, Newton (15) has presented some very striking data to show the protective action of sugar against the precipitation of proteins in expressed plant saps.

SUMMARY

1. A study has been made of the seasonal variations in the sugar content, the water content, and the volume of water expressed as sap under pressure after various treatments for the leaves of the pitch pine. Determinations have also been made of the seasonal variations in the osmotic value of the sap expressed from the leaves of this species.

2. The total water content of the mature leaves of the pitch pine does not exhibit any marked variations. It is highest in the recently developed leaves during the first summer of their existence. The water content decreases very slowly during the autumn and winter months until April or May and rises slightly during the following summer months. The lowest leaf water contents occur in the late spring at a season when the leaves are seldom subjected to freezing temperatures.

3. Since the water contents of the hardened leaves during the winter months are not materially less than the water contents of the unhardened leaves during the following summer, a decrease in the moisture content of the leaf tissues is not a factor in the winter cold resistance of the leaves of this species.

4. During the summer months the leaves of the pitch pine are readily killed by freezing in an ice-salt bath and the sap easily expressed. In the winter the yield of sap from leaves treated in the same way is much smaller, showing that the leaf cells are not killed by the freezing process. The water-retaining capacity of the leaf cells of the pitch pine is therefore greater in the winter than during the summer, resisting the forces of crystallization operating in the intercellular spaces, which in the summer are able to withdraw sufficient water from the interior of the cell to cause disorganization of the protoplasm. The increased water-retaining capacity of the cells in the winter is believed to be due to an accumulation of colloidal gels which increase the proportion of bound water in them during the late autumn months. The colloidal content of the leaf cells is also believed to decrease in the late spring, accounting for the decrease in the water-retaining capacity of the tissues which occurs at that season.

5. A freezing and high-pressure treatment of leaf samples is described which appears to furnish a relatively simple and adequately quantitative method for distinguishing between bound and free water in leaf tissues.

6. Unfrozen samples of the leaves of the pitch pine show a greater resistance to dehydration under pressure in the winter than in the summer. This is regarded as contributory evidence for the view that there is an accumulation of colloidal gels in the leaf cells during the winter months, since it is to be expected that tissues in this condition would show a greater resistance to dehydration under pressure than tissues in which only a relatively small amount of the water was bound by imbibitional forces.

7. It is believed that the seasonal variation in the relative proportions of bound and unbound water is the most important factor in the cellular physiology of the leaves of this species in relation to cold resistance. That this seasonal variation in the proportion of bound water is due primarily to the seasonal changes in the amount and condition of the cell colloids appears to be reasonably well established.

8. The osmotic value of the expressed leaf tissue fluids of this species is, in general, higher during the winter months than during the other seasons but the seasonal range is not great. The maximum value recorded was 25.73 atmospheres (January 1928).

9. The low winter values for the osmotic pressure lead to the conclusion that this is only a minor factor in the cold resistance of pitch pine leaves. Since, however, it seems certain that the proportion of water available for solution is reduced in the winter, it is conceivable that the winter osmotic pressure of the cell sap may be much higher than determinations of the osmotic value of the expressed leaf saps seem to indicate, and, therefore, of greater significance than the available experimental data seem to show.

10. If reliable osmotic data are to be obtained on expressed leaf saps during the winter months, a sufficiently drastic method of treatment must be employed to destroy the colloidal complex of the hardened leaf cells. Grinding, and freezing at the temperature of solid carbon dioxide are methods which have been employed successfully in the present investigation for this purpose. Samples of leaves in the winter condition, frozen in an ice-salt bath, do not yield representative samples of sap under pressure.

11. The sugar content of the leaves of the pitch pine increases during the autumn months, is relatively high during the winter, decreases during the spring, and is relatively low during the summer.

12. The increase in soluble carbohydrates during the winter months is undoubtedly the important factor in causing the increase in the winter osmotic values of the saps expressed from these leaves. This accumulation of sugars may also be of importance in the cold resistance of pitch pine leaves through the protective action which sugars exert against precipitation of proteins.

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OBSERVATIONS ON THE FEEDING HABITS OF THE SWARM CELLS OF MYXOMYCETES¹

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INTRODUCTION

The Myxomycetes are a group of small, rather inconspicuous organisms having affinities with both the plant and animal kingdoms, and their study, which has been chiefly taxonomic, has developed almost entirely during the last half century (8, 9, 13). Early writers were entirely unaware of their true nature and thought them Gasteromycetes because of the superficial similarity to that group. Fries in 1829 (3) realized that they were not "puff balls" and created a new sub-order, the Myxogastres, to contain them, although still including them in the Gasteromycetes. It was not until 1858, when DeBary (1) worked out their remarkable life history and found that the spores gave rise, not to mycelium but to swarm cells which later united to form the plasmodium, that the Myxomycetes or Slime Moulds were removed from the Gasteromycetes and considered as a separate group, unrelated to the higher or filamentous fungi.

On the feeding habits of their vegetative swarm cells, little information has been obtained by previous investigators, although the methods by which the plasmodium obtains its food have been more carefully worked out. Previous to 1890, when Lister (7) proved that bacteria were ingested, the swarm cells were thought to obtain all of their nutriment in solution. Since that time, Lister and others have shown that bacteria constitute a considerable part of their food, and recently, in a study of this point, the writer (5) has found that the swarm cells of *Dictydiaethalium plumbeum* are able to take in and digest not only bacteria, but also the spores of certain filamentous fungi. It was the purpose of the present investigation, therefore, to determine whether the ability of the swarm cells of Myxomycetes to ingest fungous spores is general throughout the group and, if so, to find on how great a diversity of species of filamentous fungi they would feed.

MATERIALS AND METHODS

The myxomycetous spores were sown in distilled water in Syracuse glasses and the feeding habits of the swarms observed by means of a water-immersion lens. The spores of the Myxomycetes and of the fungi that were to be tested as possible food were usually sown together, but if

¹ Contribution No. 97 from the Cryptogamic Laboratories of Harvard University.

the latter were of a type that soon gave rise to mycelium, it was necessary to sow the spores of the myxomycete first and, as soon as they germinated, to add the spores of the fungus. About twenty species of Myxomycetes which had been gathered in Massachusetts during 1924-1926 (4) were used in these experiments, every important family being represented by at least one species. The filamentous fungi were from many sources, and since care was taken to obtain as many as possible from old logs and other situations where Myxomycetes were abundant, spores from species of the Agaricaceae, Hydnaceae, Polyporaceae, Mucoraceae, Discomycetes, Pyrenomycetes, and the various families of the Hyphomycetes were well represented. In addition to the spores of the fungi mentioned above, others of the Mucoraceae and Hyphomycetes were used even though the swarm cells would have been unable to come in contact with them in nature. These spores were from stock cultures used for laboratory study and also from cultures of fungi known to be pathogenic to men and animals.

The behavior of the swarm cells was recorded by camera lucida drawings and micro-photographs (Pls. XXX, XXXI), the latter being taken with a Leitz "Makam" Mikraufsatzkamera. The swarm cells, for the most part, were killed with osmic acid and lightly stained with Neutral Red and then were either photographed in the Syracuse glasses or were pipetted on slides and photographed.

GENERAL CONSIDERATION OF FEEDING HABITS

The swarm cell phase, which forms the subject of this investigation, occupies but a small part of the typical life history of a myxomycete, which under natural conditions is as follows: the spores are disseminated for the most part by the wind and by the washing of the rain, and under suitable conditions of warmth and moisture germinate, forming unicellular, free-swimming swarm cells. These divide by fission and move about actively by two methods of locomotion that grade into one another and often alternate; the first is an active rotating movement and the second a slower, more or less undulatory, creeping movement. After a day or so of such activity, the swarm cells withdraw their flagella and move entirely by means of pseudopodia. During this stage, in which they are known as myxamoebae, they also divide by fission. If the active swarm cells or myxamoebae be subjected to adverse conditions such as dryness, each forms a wall around itself and becomes a microcyst, resuming its activities, however, if conditions again become favorable. Eventually the myxamoebae unite in pairs to form zygotes and the zygotes creep together in groups to form the plasmodium. The plasmodium feeds and grows, its nuclei increase in number by division, and finally, under favorable conditions, fructifications are formed in which the spores are developed, and the life history is completed.

Feeding, however, does not take place during the whole of the swarming

period just mentioned. During the rotating movement, the posterior end of the body of a swarm cell is more or less rounded with no apparent pseudopodia (Pl. XXX, figs. 1 *d*, 4 *c*, 7 *c*). The writer has never seen a swarm cell ingest bacteria or spores while in this condition nor has he found evidence in the work of others to indicate that this occurs. During the creeping or undulatory movement, however, the posterior part of a swarm cell is not always rounded and often puts out tenuous pseudopodia (Pl. XXX, figs. 2 *b*, 3 *a*, 5 *b*, 6 *a*, 8 *b*, 9 *a*). These are usually not distinct unless stained, but their position may be inferred from the movement of particles to which they attach themselves. In taking solid matter into the body, one or more of these delicate pseudopodia become fastened to a particle and are then retracted, drawing it toward the body where extensions of protoplasm fold over and enclose it. This method of ingestion is thus slightly different from that of myxamoebae and plasmodia which ingest spores and bacteria by extending a part of the body and surrounding or engulfing them.

When ingested, the object becomes enclosed in a vacuole and may remain in any part of the cell with the exception of the anterior region near the nucleus, but is frequently moved to various places in the body during the activities of the organism (Pl. XXXI, figs. 10–16). Unless expelled earlier, the object, after a number of hours, becomes smaller and more indistinct and is digested, with the exception of unassimilable parts such as oil globules, which are thrown out. These are brought to the surface of the protoplasm in a vacuole, and for the most part are merely voided in the water by its contractile action; but occasionally they may be expelled almost explosively as the vacuole suddenly empties itself. In an old culture containing spores with oil globules (Pl. XXX, fig. 9 *c*), a number of these globules may be seen floating free, sometimes with ragged remnants of the spores attached to them (Pl. XXX, fig. 9 *d*).

While spores of many species of fungi are ingested by the swarm cells, the latter seem to find especially suitable as food and to ingest voraciously the spores of certain species such as *Monilia candida* Bon., *Isaria felina* (DC.) Fr., and *Diaporthe oxyspora* (Pk.) Sacc. (conidia); and in a culture of swarm cells which had contained many of these spores, after two weeks or more often only the swarmers or their microcysts alone were left, the fungous spores having been entirely consumed. When in such cultures, the swarm cells often become filled with the fungous spores in all stages of digestion (Pl. XXX, figs. 3 *a*, 6 *a*, 9 *a*; Pl. XXXI, figs. 10–12, 14–16), growing very large and occasionally assuming, temporarily, more or less abnormal shapes. During the digestion of the spores, they move sluggishly and often withdraw their flagella for a time (Pl. XXX, figs. 6 *b*, 8 *c*). Division is frequent and the swarm cells do not necessarily throw out any partly digested spores in preparation for the process but retain them, so that each daughter cell may receive part of the original number (Pl. XXX, fig. 6 *c*). Sometimes

division of the nucleus takes place but the body does not completely divide until later, so that for a short period the body is double and has a geminate appearance.

Spores of some species however, although ingested readily, do not appear to be digested and are sooner or later thrown out, while inorganic matter such as particles of carmine and silica is occasionally taken in by the swarm cells but is treated as inert matter and is also promptly ejected.

The spores of many species of fungi are too large for the swarm cells to ingest, for, as has been shown by the writer (5), the relation of the volume of the swarm cell to that of the spore which it can ingest is about five to one (Pl. XXXI, fig. 13). These large spores, however, may be taken in by zygotes and plasmodia, while the plasmodium, as is well known, ingests hyphae and other large particles of organic matter indiscriminately.

FEEDING ACTIVITIES WITH RELATION TO THE FUNGI USED AS FOOD

In procuring fungous spores with which to feed the swarm cells, a large number of fungi of all the main groups were tried without making any restricted choice, except that as many of the lignicolous fungi as possible were used. Thus, six species in the Phycomycetes, eight in the Ascomycetes, six in the Basidiomycetes, and twenty-two in the Imperfecti were tried as food. On a basis of the method by which the spores had been taken in by the swarm cells of *Dictydiaethalium plumbeum*, the former had previously been divided by the writer (4) into four convenient categories as follows: (1) spores that are easily ingested and digested; (2) spores that are ingested in quantities but do not seem to be digested; (3) spores that are too large to be taken in; (4) spores of *Penicillium*, *Aspergillus*, *Stysanus*, and similar genera, which are apparently unfavorable to the swarm cells and are practically never taken in. Although all of the fungi used in the experiments fall into one or the other of these four categories, it seems easiest, for convenience and clarity, to consider them in the following discussion according to their natural classification.

Bacteria

Bacteria are readily taken in and digested by the myxomycetous swarm cells (Pl. XXXI, figs. 10-12, 14-16), a fact first reported by Lister in 1890 (7), while Pinoy (10, 11, 12) even went so far as to say that bacteria not only are necessary for the existence of the swarm cells but also are essential for the germination of the spores. Although the validity of the latter theory is questionable, there is no doubt that bacteria form a considerable part of the food of the swarmers.

Myxomycetes

Inasmuch as many Myxomycetes occur in close proximity in nature, it seems possible that swarm cells might ingest each other. This has never

been observed, but zygotes and plasmodia have often been seen to ingest microcysts and ungerminated myxomycetous spores.

Phycomycetes

Among Phycomycetes, the Mucorales are most commonly found on substrata where Myxomycetes are present. Six species were sown in cultures of myxomycetous swarm cells and the spores of two, *Mucor ramannianus* A. Moell. and *Circinella simplex* Van Tiegh., were readily taken in and digested. The spores of *Helicostylum piriforme* Bain., *Circinella spinosa* Van Tiegh., and *Mucor Javanicus* Wehm. were taken in in smaller numbers while the spores of *Rhizopus oryzae* Went. were not ingested at all as they were too large (8 x 5 microns).

Ascomycetes

Eight species of Ascomycetes were used in the experiments, the stromatic Sphaeriales being represented by five species, the non-stromatic by two, and the Pezizales by one. Of the stromatic Sphaeriales, the conidia of *Endothia parasitica* (Murr.) A. & A., *Thyronectria denigrata* (Wint.) Seaver, and *Diaporthe oxyspora* (Pk.) Sacc. were sown in the cultures and all were readily ingested (Pl. XXX, figs. 2, 3, 5, 6; Pl. XXXI, figs. 15, 16), the fusiform conidia of *Diaporthe oxyspora* proving especially suitable. The ascospores of these species were too large to be ingested, all being 9-10 x 5 microns or greater. After discarding a number of other large-spored Pyrenomycetes, however, two species were obtained, *Eutypella scoparia* Schw. and *Cryptovalsa sparsa* E. & E., which had small ascospores (4 x 1 microns and 6-7 x 1-2 microns, respectively) which when sown in the cultures were readily taken in and digested.

Chaetomium cochlioides Palliser and *Chaetomium globosum* Kunze of the non-stromatic Sphaeriales were tested but they were not ingested, as might have been expected since both species had ascospores 9 x 7 microns or greater.

Of the Discomycetes, one representative species was tested, *Mollisia* sp., the ascospores of which, although comparatively large (about 7-8 x 3-4 microns), were readily taken in by the larger swarm cells.

Among the Ascomycetes, the yeasts, although not filamentous, occur where Myxomycetes abound and hence seemed suitable for experimentation. They commonly live, moreover, in moist situations where swarm cells occur, and so are occasional contaminants in myxomycetous cultures where they are easily ingested by the swarm cells, a fact previously noticed by Chrzaszcz (2) and Skupienski (14). The writer has sown, in cultures with swarmers, a number of undetermined, small-celled, wild species of the *Saccharomyces*-type, which occurred as contaminations on agar plates, and in all cases they were readily taken in and digested.

Basidiomycetes

Among the Basidiomycetes, many Hymenomycetes would undoubtedly shed spores in a locality where the swarms would be able to feed upon them. In the Hymenomycetes, spores of three species of the Agaricales were sown: *Amanita phalloides* Fr., *Collybia velutipes* Fr. (a lignicolous species), and *Cortinarius semisanguineus* Fr. The spores of *Amanita phalloides*, measuring about $9-12 \times 8-9$ microns, were too large to be ingested, as were those of *Collybia velutipes*, for the most part. However, the larger swarm cells occasionally took in spores of the latter species, which averaged $7-9 \times 3-4$ microns. The spores of *Cortinarius semisanguineus* Fr. were occasionally taken in but no digestion was observed. Probably further experiments with smaller spores of other species might give more favorable results.

In the Polyporaceae, the spores of *Fomes applanatus* (Pers.) Wallr. and *Daedalea quercina* (L.) Fr. were sown. Those of the former species were too large to be ingested but the small hyaline spores of *Daedalea quercina* were taken in in large numbers and readily digested. Both species are lignicolous and undoubtedly their spores frequently reach substrata upon which swarm cells are active.

Hydnum septentrionale Fr. of the Hydnaceae also has small hyaline spores which, like those of *Daedalea quercina*, were readily taken in and digested. As this is also a lignicolous species, occurring on logs, it is probable that a portion of its spores are consumed by swarm cells in nature.

Fungi Imperfecti

Of the Fungi Imperfecti, only members of the Hyphomycetes were used in the experiments, but in this group were included many forms, from the simple yeast-like fungi like *Monilia* to such highly complicated forms as *Isaria* and *Stysanus*. Most of the species occur normally on wood, a few were types pathogenic to animals, two were those commonly used in cheese manufacture, and a few were from stock laboratory cultures of long standing of which the original substratum is not known.

Spores of seven species were ingested in especially large numbers by the myxomycetous swarm cells, which seemed to thrive on them. These seven species, four of which are more or less yeast like, were as follows: *Candida breve* Berkhout, *Oospora humi* Mazé., *Monilia candida* Bon. (Pl. XXX, figs. 8 b, 9 a, b), *Dematium Chodati* Nech., *Acrostalagmus fragrans* Cr., *Acrostalagmus cinnabarinus* Corda., and *Isaria felina* (DC.) Fr. *Isaria felina* was especially well adapted for these experiments for the spores were sufficiently small (3×2 microns) to be ingested by the swarm cells of any of the Myxomycetes used.

*Spores of *Trichoderma lignorum* (Tode) Harz. and *Pullularia nigricans* Berkhout were taken in by the swarm cells of most species but the swarms did not ingest them as readily as they did those of the seven preceding

species, for the spores were never taken in in great numbers and the swarm cells did not become large and sluggish, as they would have had they fed upon the spores to any great degree.

The spores of a number of species were ordinarily not ingested at all. These included *Stysanus stemonitis* Cda., *Aspergillus niger* Van Tiegh., *Penicillium camemberti* Thom, *Penicillium roqueforti* Thom, and a number of undetermined species of *Penicillium* and *Aspergillus*. If the spores, however, were treated with alcohol (5), the alcohol evaporated, and the spores then sown in the cultures, they were occasionally taken in but evidently were never digested, for in all cases observed they were soon ejected, apparently unchanged.

Of those somewhat peculiar Fungi Imperfecti, which occur pathogenically on animals, usually on the skin, a number of species were used in the experiments. Of course these species in nature never come in contact with swarm cells but for this very reason, as well as because of their parasitic or semi-parasitic habits, it seemed of interest to determine the reaction of the swarm cells to them in the cultures. Of the eight pathogenic species tested, only two had spores small enough to be taken in: *Oidium cutaneum* (DeBeurm.) Goug. & Vauch. and *Actinomyces asteroides* (Eppinger) MacC. In both cases the spores were ingested, but those of *Actinomyces* were taken in in lesser numbers than those of *Oidium* although the swarm cells seemed to thrive on a diet of either.

VARIED FEEDING HABITS OF DIFFERENT SPECIES OF SWARM CELLS

Swarm cells of different species ordinarily show but slight differences in size and shape but their behavior in distilled water varies greatly and apparently has considerable influence upon their ability to ingest fungous spores. As has been stated above, the swarm cells have two distinct movements of a rotating and an undulatory type and it is only in connection with the latter that the ingestion of food has been observed.

Many species under the conditions of culture to which they are subjected in the laboratory seldom or never pass out of the rotating stage and therefore infrequently or never ingest fungous spores, although it seems probable that they might do so under different conditions in nature.

The differences in behavior of the swarm cells in culture seem entirely independent of family relationships, for the species within a family were found to vary greatly with regard to their ability to ingest fungous spores. Therefore, in the following discussion, the Myxomycetes are dealt with according to the behavior of their swarm cells rather than according to their natural classification.

From this point of view there appear to be five categories, not sharply defined, into which the Myxomycetes fall. In the first are included those Myxomycetes with swarmers that spend part of their time (a few hours to a few days, according to the species) in the rotating stage, but later change

to the creeping or undulatory stage, when of course they are able to ingest fungous spores.

Swarm cells of Myxomycetes in the second category assume the creeping stage shortly after emerging from the spore cases and hence, like those of the first group, ingest fungous spores readily, the ingestion starting very soon after the swarmers form their flagella.

As a third type may be considered certain Myxomycetes which, although they might be included in one of the two classes mentioned above, have swarm cells so small that, while they are large enough to ingest some spores, they lack the size necessary to take in such larger ones as would be ingested by swarmers of ordinary size.

A fourth class of Myxomycetes has swarm cells which under laboratory conditions almost never assume the undulatory movement but remain in the rotating stage until they encyst, and hence seldom or never take in solid food.

In the fifth category may be included Myxomycetes whose swarm cells for some reason, not as yet understood, do not ingest fungous spores to any great extent even in the creeping stage, although conditions are apparently favorable for the process.

Among the Myxomycetes of the first class whose swarm cells, after rotating for a while, pass to the undulatory stage and ingest fungous spores readily, must be included *Dictydiaethalium plumbeum*, *Leocarpus fragilis*, *Stemonitis splendens* var. *flaccida*, and *Trichia floriformis*. *Dictydiaethalium plumbeum* is perhaps the best species with which the writer has worked, since it is particularly favorable both with regard to its germination and to the ability of its swarm cells to ingest fungous spores. However, as it has been discussed in detail in a previous paper (5) it need not be given further consideration here. The swarm cells of the three other species readily ingest fungous spores but *Leocarpus fragilis* is less favorable for experimental purposes than the remaining two since its spores, on the average, take a number of days to germinate while those of the others, including *Dictydiaethalium plumbeum*, usually germinate within twenty-four hours. Division is very frequent in a culture of the swarm cells of *Stemonitis splendens* var. *flaccida* and often a culture becomes extremely crowded with rotating and creeping swarm cells. Swarmers of this and other species usually change from the rotating to the creeping movement directly, without rounding off, but occasionally in this species a swarm cell after rotating for a longer or shorter time goes to the bottom of the culture, retracts its flagellum, and remains spherical and quiescent for a short period as if it were about to divide; after which it forms a flagellum, and either starts to move by creeping or returns to its previous rotation.

In the second category should be included *Hemitrichia vesparium*, *Badhamia lilacina*, *Badhamia magna*, *Didymium nigripes* var. *xanthopus*, and *Comatricha typhoides*, since their swarm cells assume the creeping

movement soon after escaping from the spore cases. The first two of these species are not favorable for experimental work, however, as their spores seldom show a germination of over 10 percent, although the few swarm cells that do appear readily ingest fungous spores.

The swarmers of the Myxomycetes in these first two categories, after emerging from the spore cases, remain for a longer or shorter time in the rotating stage and as soon as they change to the undulatory movement ingest fungous spores, ordinarily starting with the smaller ones and progressing to those increasingly larger, in cases where the spores vary greatly in size. If the spores are of a type especially favorable for ingestion, such as *Isaria felina* or *Monilia candida*, a large number are taken in, one at a time, until the swarmer is gorged with fresh and partly digested spores (Pl. XXXI), and the process often goes on until few or no spores are left in the cultures.

In the third category must be included *Physarum viride*, *Stemonitis ferruginea*, and *Lycogala epidendrum*, all of which have swarm cells smaller ($9-10 \times 2-3$ microns) than the average. For the most part they remain in the rotating stage for a considerable period, but before becoming myxamoebae they assume the creeping stage and then are able to ingest small spores of fungi such as *Isaria felina*. Of course the myxamoebae and zygotes are able to ingest fungous spores of larger size, but so far as the swarmers are concerned it seems in these species that the small size and the short time in which they retain the creeping stage are the limiting factors in the ingestion of the spores of filamentous fungi.

In the fourth class belong *Reticularia lycoperdon*, *Enteridium splendens* (*Rozeanum*), *Fuligo septica*, and *Stemonitis fusca*, since their swarm cells seldom change from the rotating stage in culture and as a result have but occasionally been seen to ingest fungous spores.

In the fifth and last category may be included *Hemitrichia clavata*, *Arcyria denudata*, and *Arcyria incarnata* whose swarm cells ingest fungous spores only to a limited degree and seldom become gorged with them like those of the Myxomycetes in the first two classes. This fifth class is not sharply defined and is made merely to include a few species which, because of their limited ability to ingest spores, are not placed in the other classes. *Hemitrichia clavata* must be placed here because its swarmers show only a slight ability to ingest most fungous spores even though they feed voraciously on the spores of *Isaria felina*. This is, however, an unusual case, for ordinarily the swarm cells of a species do not show marked preference for the spores of any one kind of fungus.

All types of spores are not ingested, as has been shown above, and certain spores which are occasionally ingested are apparently never digested. Differences of the protoplasm probably play an important part in the variation of feeding habits of the swarm cells. In other words, those of a few species may necessarily need a certain kind of food although some

species such as *Didymium nigripes* are not so restricted as to diet (6, 14). While this specific difference in the nature of the protoplasm is in no degree so pronounced as in the rusts, it may nevertheless be sufficient to account for the failure of some swarm cells to ingest spores. On the other hand, spores of some filamentous fungi are not ingested by any of the swarmers used in the experiments. Size of spores is an important factor and the largest that are taken in are not greater than one fifth of the volume of the swarm cell (5). Of the smaller spores, those with a more or less moist or mucilaginous coat, such as the spores of the yeast-like *Monilia candida* Bon. or *Acrostalagmus fragrans* Cr., are preferred to the very dry or perhaps greasy spores of species such as *Penicillium roqueforti* Thom. As has been shown by the writer (5), spores of the latter type are only ingested after some substance, probably a layer of air or grease, has been removed by treating with alcohol. It seems probable, therefore, that the composition of the cell wall or of substances adhering to it are determining factors in the case.

Most of the spores used as food by the swarm cells were hyaline, while many of those rejected were colored, but no criterion may be established with regard to the color of the spores since some that were hyaline were not used as food while the colored spores of *Trichoderma lignorum* (Tode) Harz and *Acrostalagmus fragrans* Cr. were readily ingested by the swarm cells. All of the spores of the filamentous fungi used in the experiments had smooth walls and so it is not known whether warted, spiny, or reticulated spores are ever taken into the body of a swarm cell. There are many other factors, undoubtedly, which influence the ingestion and digestion of spores by swarm cells and this field of study has opened up a number of interesting problems for future research.

SUMMARY AND CONCLUSIONS

The ability of the swarm cells to ingest spores of higher filamentous fungi and use them as food was studied in the case of twenty representative Myxomycetes. Swarm cells were found to take in and digest spores of major groups of fungi such as the Agaricaceae, Polyporaceae, Mucorales, Pyrenomycetes, Discomycetes, and lignicolous Imperfecti, and since these spores, for the most part, came from species that occur in moist woodland situations where Myxomycetes are abundant, it seems possible that they may form a considerable part of the natural food of the swarmers.

Great variation was found in the readiness with which the swarm cells of the Myxomycetes ingested fungous spores. *Leocarpus fragilis*, *Didymium nigripes* var. *xanthopus*, *Stemonitis splendens* var. *flaccida*, *Badhamia magna*, *Badhamia lilacina*, *Trichia floriformis*, *Hemitrichia vesparium*, *Comatricha typhoides*, and *Dictydiaethalium plumbeum*, for example, ingested spores of nearly any of the fungi tested, provided they were not too large, and hence proved to be the best for experiments on feeding. On the other hand,

Fuligo septica, *Stemonitis fusca*, *Enteridium splendens* (Rozeanum), *Hemitrichia clavata*, *Arcyria denudata*, *Arcyria incarnata*, and *Reticularia lycoperdon* did not take in spores in nearly as large amounts as other species, possibly because their swarm cells in culture spent most of their time in the rotating stage, during which it was found that ingestion did not take place. A number of Myxomycetes such as *Physarum viride*, *Stemonitis ferruginea*, and *Lycogala epidendrum* were found to have swarm cells smaller than the average and therefore were unable to ingest very large spores. Ability to ingest smaller spores, however, showed that size rather than any specific reaction to the chemical nature of the bodies was the limiting factor in these cases.

With few exceptions, spores that were vigorously ingested by swarm cells of one myxomycete were ingested by all, although by some in lesser numbers, and spores not taken in by one were not taken in by the others; in other words, no myxomycete showed a special preference for spores which were not taken in to a greater or less degree by other Myxomycetes. No family showed a marked ability to ingest spores, this characteristic appearing to be entirely specific.

The writer is greatly indebted to Dr. Lewis E. Wehmeyer for authentic cultures of the Pyrenomycetes used in the experiments; and for aid and criticism he wishes to express his gratitude to Dr. William H. Weston, Jr., under whose supervision the work was carried out.

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EXPLANATION OF PLATES

PLATE XXX

The original ink drawings have been reduced from 12 × 8 inches. The camera lucida outlines were made with a Leitz * D water-immersion objective, and ocular No. 15, and the swarm cells, immediately before being drawn, were killed by the addition of a drop of osmic acid to the water in the Syracuse glasses.

FIGS. 1-3. Stages in the ingestion of the conidia of *Thyronectria denigrata* by the swarm cells of *Leocarpus fragilis*. × 800.

FIG. 1. *a*, spore of *Leocarpus fragilis*; *b*, germination of spore; *c*, swarm cell before formation of flagellum; *d*, young swarm cells.

FIG. 2. *a*, spore of *Leocarpus fragilis* still containing one of the original swarm cells; *b*, young swarm cells starting to ingest the conidia of *Thyronectria denigrata*; *c*, conidia of *Thyronectria denigrata*.

FIG. 3. *a*, swarm cells stuffed with conidia undergoing digestion; *b*, free conidia of *Thyronectria denigrata* for comparison.

FIGS. 4-6. Stages in the ingestion of the conidia of *Diaporthe oxyspora* by the swarm cells of *Stemonitis splendens* var. *flaccida*. × 800.

FIG. 4. *a*, spores of *Stemonitis splendens* var. *flaccida*; *b*, empty spore case; *c*, young swarm cells after emergence.

FIG. 5. *a*, empty spore case; *b*, swarm cells starting to ingest conidia of *Diaporthe oxyspora*; *c*, conidia of *Diaporthe oxyspora* for comparison.

FIG. 6. *a*, swarm cells stuffed with conidia; *b*, swarm cell which has temporarily withdrawn its flagellum during the process of digestion of conidia; *c*, swarm cell undergoing division, showing that ingested food material is not cast out during the process; *d*, conidia of *Diaporthe oxyspora*.

FIGS. 7-9. Stages in the ingestion of spores of *Monilia candida* by the swarm cells of *Dictydiaethalium plumbeum*. × 800.

FIG. 7. *a*, spores of *Dictydiaethalium plumbeum*; *b*, empty spore case; *c*, young swarm cells.

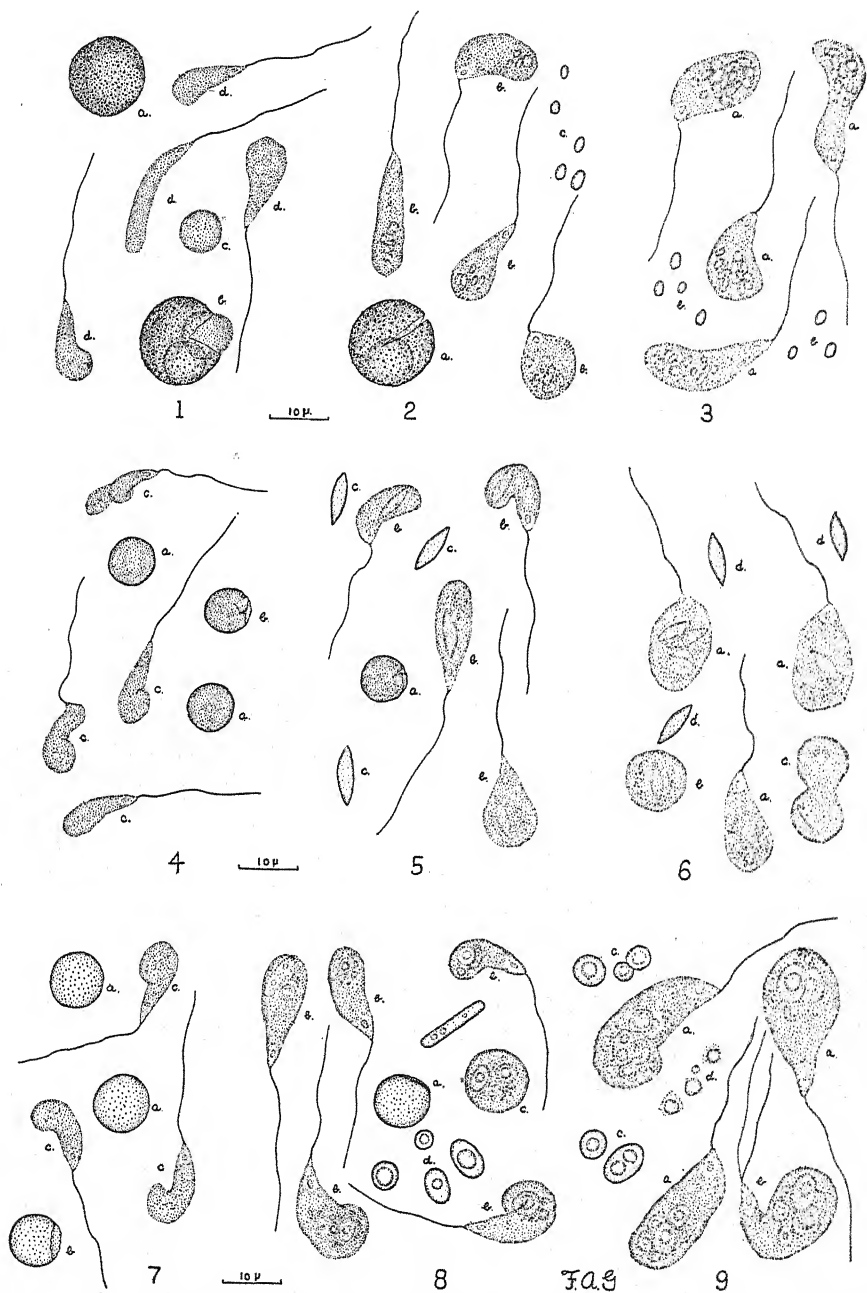
FIG. 8. *a*, empty spore case; *b*, swarm cells with ingested spores; *c*, swarm cell which has temporarily withdrawn its flagellum during the process of digestion of fungous spores; *d*, spores of *Monilia candida* for comparison.

FIG. 9. *a*, swarm cells engorged with spores of *Monilia candida*; *b*, engorged swarm cell with two flagella; *c*, spores of *Monilia candida*; *d*, oil globules expelled by the swarm cells after the digestion of the remainder of the spores which formerly enclosed them.

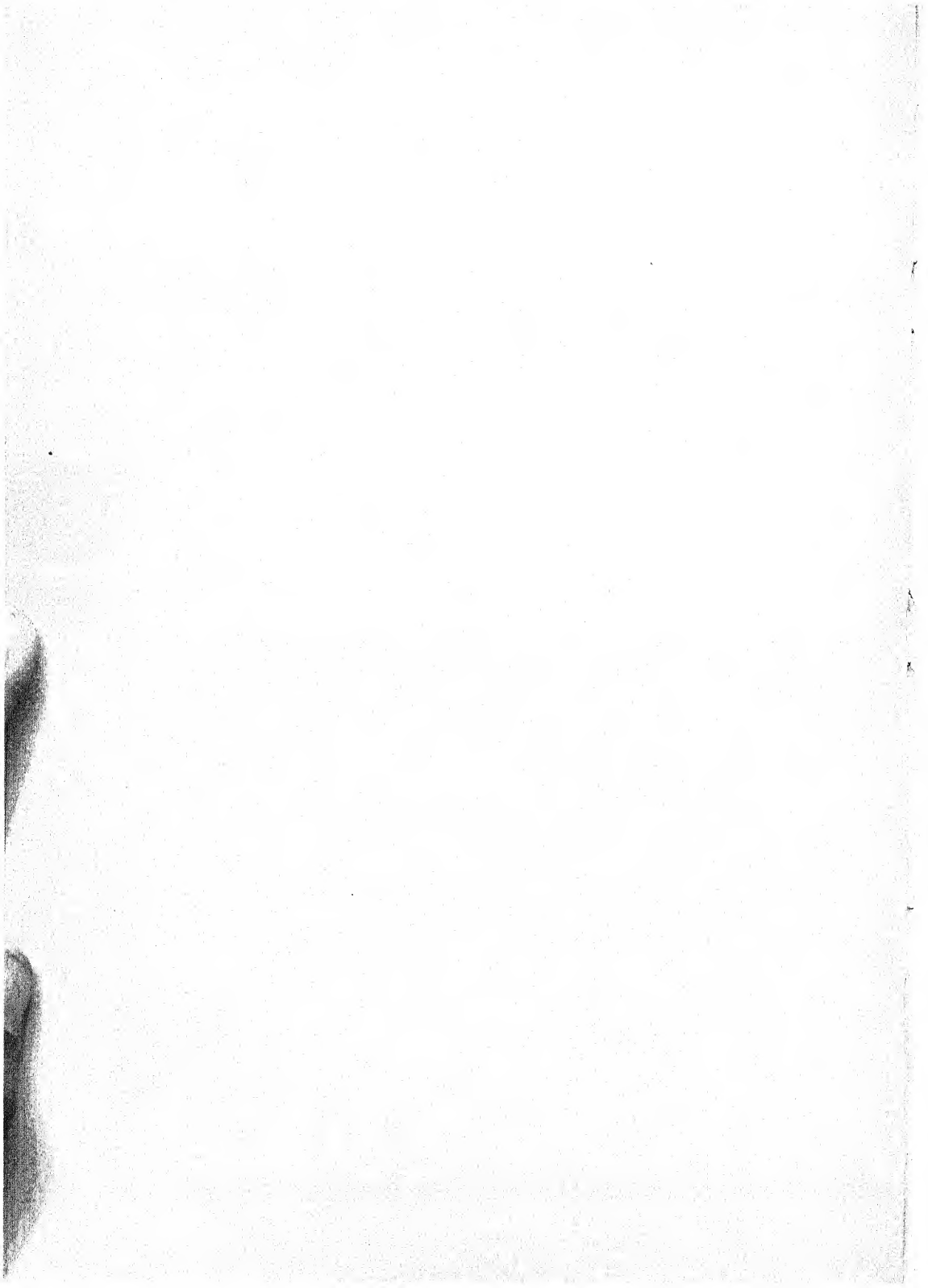
PLATE XXXI

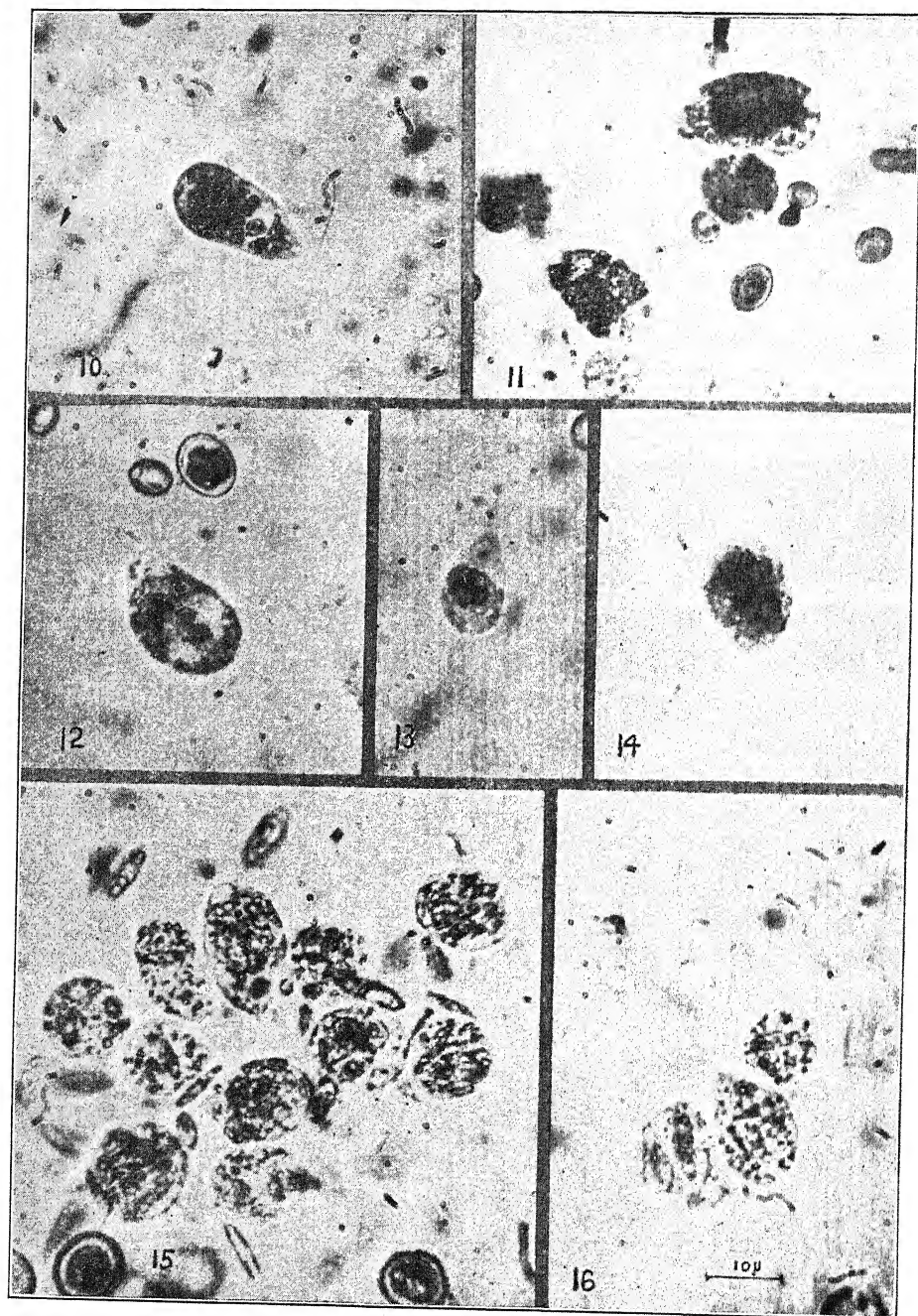
FIGS. 10-14. Swarm cells of *Dictydiaethalium plumbeum* engorged with bacteria and with spores of *Monilia candida*. × 1,000.

FIGS. 15-16. Swarm cells of *Dictydiaethalium plumbeum* engorged with bacteria and with conidia of *Diaporthe oxyspora*. × 1,000.



GILBERT: FEEDING HABITS OF SWARM CELLS





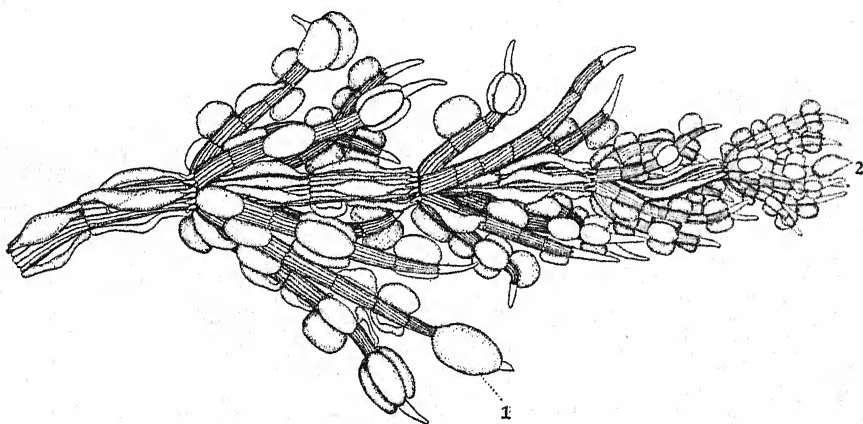
GILBERT: FEEDING HABITS OF SWARM CELLS

STUDIES IN THE CHYTRIDIALES III. A PARASITIC CHYTRID CAUSING CELL HYPERTROPHY IN CHARA

J. S. KARLING

(Received for publication April 6, 1928)

In the summer of 1924 I received two cultures each of *Chara contraria* and *C. delicatula* from Dr. S. P. Nichols of Oberlin College which had been collected in the town reservoir at Oberlin, Ohio. These cultures were subsequently grown for several months in tap water in New York City, and during this time the corticating cells surrounding the internodes of stems and leaves, the leaflets, stipules, pro-embryonic branches and the internodes themselves became enormously hypertrophied and distorted. The plants had the appearance of being covered with large green oblong



TEXT FIG. 1. Terminal portion of a plant of *C. contraria* which has from one to several hypertrophied corticating cells on each internode of the stem and leaves. 1 and 2, hypertrophied end segments of leaves.

and round blisters (text fig. 1). In the majority of plants affected the diameter of the swollen corticating cells was greater than that of the entire internodal cell. Examination of these plants showed that the hypertrophied cells contained numerous oblong, round, or disk-shaped bodies of various sizes which were passively carried along with the host nuclei in the streaming cytoplasm, while adjacent normal cells were entirely free of bodies of this kind. These bodies were at once recognized as an intracellular parasite, but before its life cycle could be completely worked out the epidemic sub-

sided; and the plants which were not wholly killed recovered and continued to grow and fruit. The same species of *Chara* have been repeatedly secured from the reservoir at Oberlin and grown in New York City tap water, but no further indications of the disease have appeared in the new cultures. In view of my inability during the past four years to obtain additional material for the study of this very characteristic disease it seems worth while to give a preliminary account of it with figures of the stages which I have so far observed.

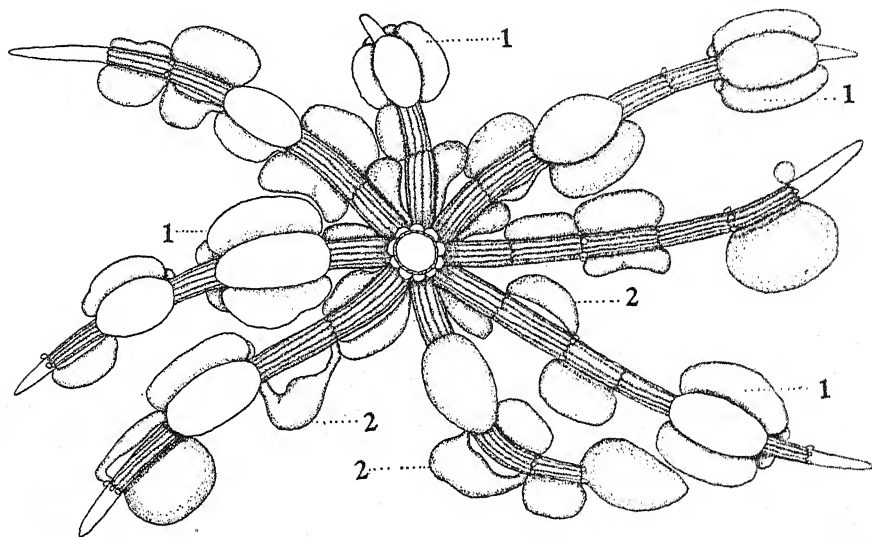
The life history of the causal organism as far as is at present known appears to consist of two distinct stages. The first stage occurs in parasitized host cells which have just begun to swell, and consists of round and oblong, somewhat disk-shaped multinucleated bodies which vary from $14\ \mu$ to $90\ \mu$ in their greatest diameter and lie naked in the primordial utricle of the host cell. In appearance and structure these bodies bear some resemblance to the multinucleated plasmodial stage of certain Monadineae, the parasitic slime moulds, and certain members of the Synchytriaceae or Merolpidiaceae (Fischer, 1892). Multinucleated, naked, more or less amoeboid-shaped plasmodia are characteristic of the parasitic Myxomycetes, Monadineae, and certain Synchytriaceae, but diagnostic characteristics at this stage are so far lacking. The nuclei are generally typical and easily fixed in nearly all of these forms, and this is likewise true of the parasite on *Chara*.

The second stage follows as the host cells continue to swell. It consists of an aggregate body or sorus somewhat similar in size and shape to the bodies described above. The sori are generally round and oblong disks, $15\ \mu$ to $70\ \mu$ in diameter, and are made up of from one to over one hundred sporangium-like cells. In appearance and structure they are strikingly similar to the sori of the Synchytriaceae or Merolpidiaceae, particularly *Woronina* and *Rhizomyxa*. Superficially they resemble somewhat the spore balls of the parasitic slime moulds, such as *Sorosphaera*, *Spongospora*, *Sorodiscus*, *Ligniera*, etc. The sporangium-like cells which compose the aggregates or sori vary greatly in size and shape, and each has from one to several thickened caps, collars, or opercula on their walls. In this respect they are similar, on the one hand, to the spores of *Sorodiscus* (Winge, 1912, fig. 70) and on the other hand to the sporangia of *Physoderma* and *Cladochytrium*. The second stage of the organism thus has characteristics which are similar in many respects to those of the Monadineae, parasitic Myxomycetes, Synchytriaceae, and Cladochytriaceae.

SYMPTOMS OF THE DISEASE

The most striking symptom of the disease on the *Chara* plant is an extreme hypertrophy of the cells affected. All cells of the plant appear to be equally susceptible; hypertrophied stipules, leaflets, spicules, and internodal cells as well as swollen corticating cells have frequently been

found. In the majority of plants affected, however, the corticating cells of the stems and leaves were more often attacked. A few pro-embryonic branches were found whose uncorticated cells were swollen, and in several mature plants the disease had penetrated into and caused hypertrophy of the internodal cell. The extent to which a plant may become infected and distorted is shown in text figure 1. Such plants when observed with the unaided eye appear to be covered with oval and elongated green blisters or balloon-like cells. In this figure, which represents an extreme case of infection, nearly every internode of the stem and leaves has several swollen and distorted cells. In the internodes of the leaves the short cor-

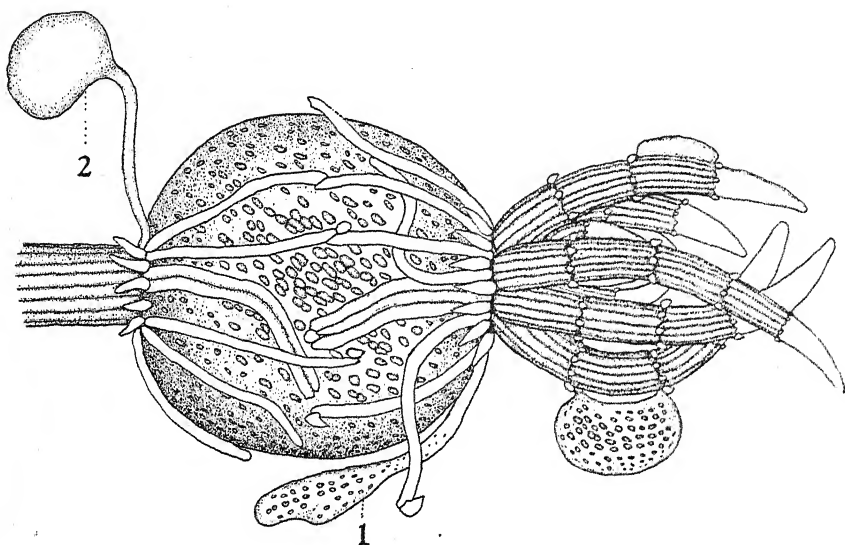


TEXT FIG. 2. A single whorl of leaves of *C. delicatula* with several hypertrophied corticating cells on each leaf.

ticating cells are rounded, while in the internodes of the stem they are more cylindrical and elongated. This difference is well illustrated in figures 1, 2, and 3, Plate XXXII. Frequently the uncorticated end cell of a leaf may become infected and swell to the enormous size shown at 1 and 2 in text figure 1.

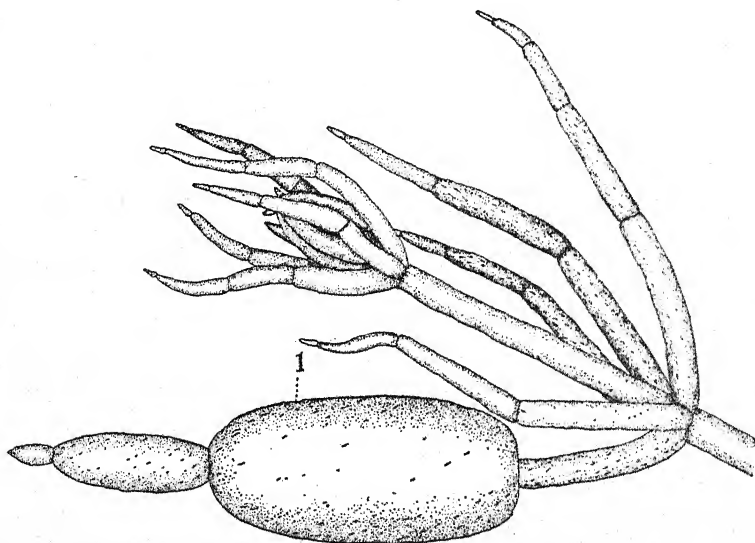
In text figure 2 is shown a single whorl of leaves which represents the most extreme case observed in either *C. contraria* or *C. delicatula*. Every leaf in this whorl has from five to nine hypertrophied corticating cells, and on each of the four leaves numbered 1 in this figure are five swollen cells around one internode, forming peculiar structures which resemble groups of carpels. As is shown in the cells numbered 2, the corticating cells frequently separate from the internode as they begin to swell. Such cells may be readily removed from the internode and cultured apart from the

plant for a considerable length of time. In figure 4, Plate XXXII, are shown short corticating cells which were removed from a leaf and kept



TEXT FIG. 3. A swollen internodal cell of *C. delicatula* which has burst the surrounding sheath of corticating cells. The tips of two of the corticating cells, 1 and 2, are likewise swollen.

alive for ten days in a culture of tap water. Up to the time the cells died the host cytoplasm, nuclei, and bodies circulated continuously in the cells.



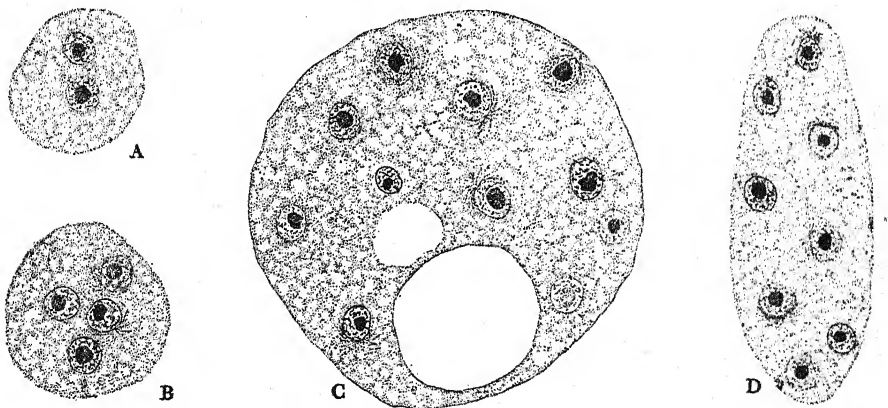
TEXT FIG. 4. Terminal portion of a pro-embryonic branch of *C. contraria* with three hypertrophied internodal cells.

The extent to which an internodal cell may swell when parasitized is well illustrated in text figure 3. This figure shows the apical end of a plant in which the terminal internode has swollen into a sphere-like cell whose diameter is fully four times that of the normal internode below. The extreme bulging of the internode has burst the sheath of corticating cells and caused them to separate, so that they appear as a fringe around the distended cell. Two of the separate corticating cells, marked 1 and 2, are likewise hypertrophied, but unlike the cells shown in figure 2, Plate XXXII, the swelling is confined to their tips.

The development from the nodes of old plants of so-called pro-embryonic branches, whose internodes are at first wholly uncorticated, is a common occurrence in the Characeae. The cells of such branches have very thin walls and are very susceptible to attack. Text figure 4 shows the terminal portion of a pro-embryonic branch in which three of the uncorticated internodes of a leaf have become infected and hypertrophied. The cell numbered 1 in this figure is fully four times as large in diameter as the internode below it. The two internodes above are also parasitized but only slightly swollen.

LIFE HISTORY OF THE CAUSAL ORGANISM

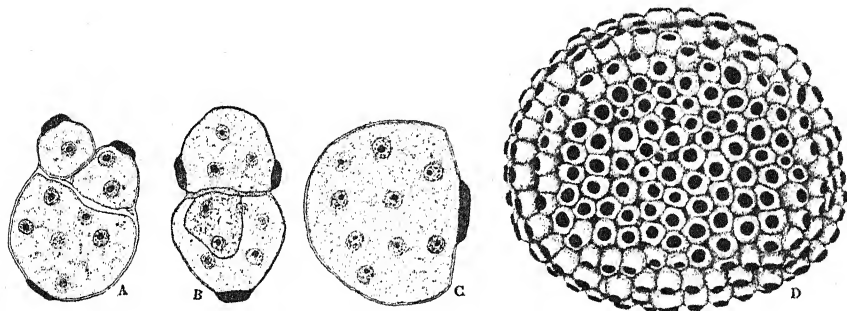
When the hypertrophied *Chara* cells are examined under the microscope numerous bodies of various sizes and shapes may be seen floating in the streaming host cytoplasm. In cells which have recently begun to swell the bodies are rounded and oblong, somewhat flattened, disks, 14μ to 90μ in their greatest diameter, as is shown in text figures 5 and 7 and figures



TEXT FIG. 5. A and B, early stages in the development of the naked bodies. C and D, surface and edge views of the same type of bodies.

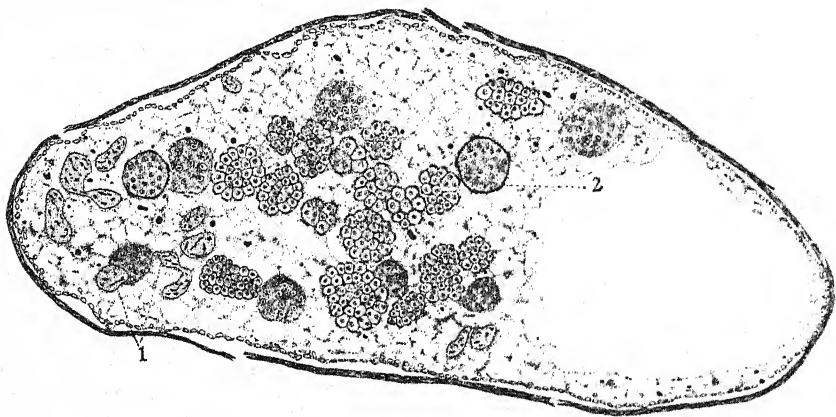
7 and 8 of Plate XXXII. The last two are photographs of the bodies in the living condition, in which the nuclei appear as darker spots. These bodies are multinucleated and lie naked in the host cytoplasm, and in these

respects they appear in general like the multinucleated stages of *Sorosphaera* (Maire and Tison, 1909, figs. 18, 22, 29, 30, 34, 36, and 37; Schwartz, 1910, figs. 18 and 19; Schwartz, 1911, fig. 9), *Ligniera* and *Molliarda* (Maire and Tison, 1911, figs. 24, 27, 62, and 63), *Spongospora* (Osborn, 1911, figs. 6, 12, and 13), *Sorolpidium* (Nemec, 1911, figs. 3, 29, and 30), *Sorodiscus* (Winge, 1912, figs. 11 and 64), *Olpidiopsis* (Barrett, 1912, figs. 32, 34, 36, and 37), and *Diplophysalis* (Cienkowski, 1865, and Zopf, 1885). A large vacuole



TEXT FIG. 6. A, B, and C, sporangiospores composed of from one to several cells. D, a sorus composed of approximately 200 cells.

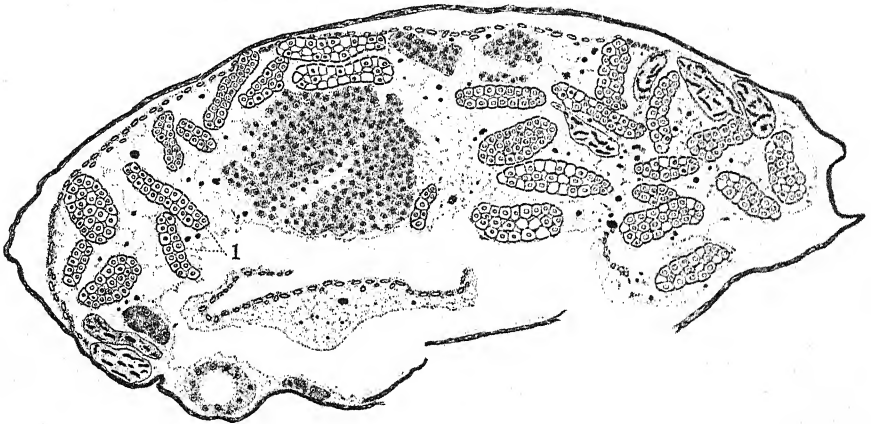
is frequently present in the center of the body or slightly displaced towards the periphery (text fig. 5 c).



TEXT FIG. 7. A longitudinal section of a cell of *C. contraria* with numerous naked bodies and sporangiospores lying intermingled with the host nuclei.

Text figures 5 a and 5 b show what appear to be two- and four-nucleated stages in the development of these bodies. So far a uninucleated zoospore or amoeba stage has not been found. Numerous unciliated and uninucleated zoospores have been found in old hypertrophied cells in connection with these bodies, but a careful study of their progressive

development showed them to be the zoöspores of *Entophlyctis heliomorpha*, *Diplophlyctis intestina* (Karling, 1928 *a* and 1928 *b*), *Diplophysalis nitellarum*, *D. stagnales* (Cienkowski, 1865, and Zopf, 1885), and *Pseudospora nitellarum* (Cienkowski, 1865), which are frequently associated with this organism. Whether the binucleated and multinucleated stages have arisen by the fusion of uninucleated zoöspores or amoebae as in the Mycetozoa, or by the division and growth of uninucleated amoebae as in the chytrids, is not yet clear. Numerous eight- and sixteen-nucleated stages have been found, and the bodies appear to increase in size with the multiplication of the nuclei until they frequently reach a diameter of $90\ \mu$. Text figure 8 shows a large body with more than a hundred nuclei. The body shown in this figure, however, is irregular and amoeboid in shape and suggests in appearance a plasmodium. However, among the hundreds of specimens observed very few of this size and shape have been found.



TEXT FIG. 8. A fairly old swollen cell of *C. delicatula* with numerous sporangiosori and host nuclei in the cytoplasm. Several sporangiosori appear in side view.

No amoeboid movement of the bodies has yet been observed. However, such movement may be difficult to see, since in living host cells the bodies are carried along quite rapidly in the streaming cytoplasm. They may well be plasmodia which as a result of their unusual environment are generally rounded in shape and rarely show amoeboid movement. The plasmodia of the Myxomycetes and the early stages of the sporangium of the Olpidiaceae and certain members of the Synchytriaceae are generally naked and lacking in a thick surrounding membrane, and in this respect these bodies resemble them. However, it seems probable that they later become invested with a wall as in *Olpidiopsis*, *Woronina*, and *Anisomyxa*, undergo cleavage, and become transformed into the sporangiosori or aggregates to be described below.

As the infected *Chara* cells continue to swell and grow old another body of quite different appearance is found in the cells in addition to the few-

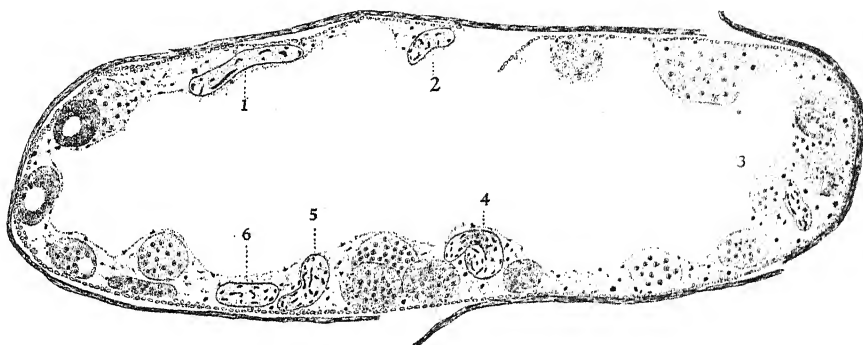
and many-nucleated masses just described (text fig. 5 and Pl. XXXII, fig. 6). This is an aggregate made up of smaller bodies or flask-shaped cells. Figure 9, Plate XXXII, shows such a sorus more highly magnified. Although it is not certain that these bodies are aggregations of sporangia, I shall call them *sporangiosori*. As many as four hundred such sori have been found in a single large *Chara* cell. The sporangiosori have the appearance of flattened disks (Pl. XXXII, fig. 9), $15\ \mu$ to $70\ \mu$ in their greatest diameter, and may be composed of from one to over a hundred cells (text figs. 6 *c* and 6 *d*). Text figure 8 shows a number of sporangiosori in edge view, and it is evident from this figure, particularly in the region marked *I*, that they may often be but two cells in thickness. The sori here shown are strikingly similar to the side view of a spore cake of *Sorodiscus* as it is figured by Winge (1912, fig. 63).

The appearance and structure of these aggregates or sporangiosori are very similar to the resting sporangiosori of *Woronina* (Cornu, 1872, figs. 15, 16, 17, and 18, pl. 7; Fischer, 1882, figs. 15 and 17), *Rhizomyxa* (Borzi, 1884, and De Wildeman, 1893), *Pyrrosorus* (Juel, 1901, fig. 1), and *Anisomyxa* (Nemec, 1912). In these genera of the Synchronaceae or Merolpidiaceae the resting sporangia are grouped together in sori of various sizes and shapes and surrounded individually by a fairly thick wall. These sporangiosori are in some respects perhaps similar to the spore balls of the parasitic slime moulds, such as *Sorosphaera* (Maire and Tison, 1909, figs. 53 and 65; Schwartz, 1910, figs. 14 and 15; Schwartz, 1911, figs. 14 and 20), *Spongospora* (Osborn, 1911, fig. 16), and *Ligniera* (Maire and Tison, 1911, figs. 42 and 45).

The sporangium-like cells which compose the sporangiosori are spherical when occurring singly, and angular in outline when pressed together (text fig. 6 *d* and Pl. XXXII, figs. 10 and 12). In this latter respect they look very much like the resting sporangia of *Woronina* as they are figured by Fischer (1882, fig. 16). They contain, like the sporangia of *Anisomyxa* and *Sorolpidium* (Nemec, 1911 and 1913), from one to many nuclei according to their size (text figs. 6 *a*, 6 *b*, and 6 *c*). Their walls are comparatively thick and have from one to several circular differentiated regions which agree in many respects with caps or opercula of the sporangia of *Physoderma* (Tisdale, 1919) and *Cladochytrium* (Clinton, 1902) and the spores of *Sorodiscus* (Winge, 1912, fig. 70). These cells may be readily dissociated. Figure 10, Plate XXXII, shows four in surface view, and this figure is very similar in superficial appearance to a surface view of a spore cake of *Sorodiscus* as figured by Winge (1912, fig. 62). The caps or opercula appear as dark circular disks or plates. Figure 11 shows a cell in side view, which is quite like the uninucleated spore of *Sorodiscus* shown by Winge in figure 70. Figure 12 shows the same cell as seen from above. Due to mutual pressure in the sorus this cell is markedly vase-shaped.

HOST AND PARASITE RELATIONSHIP

The sporangesori and the naked multinucleated bodies lie in the primordial utricle of the host cell and are completely surrounded by its cytoplasm. In text figure 9 are shown 18 naked bodies in the primordial utricle of a young cell with six host nuclei, and in text figures 7 and 8 optical sections of slightly older cells with both types of bodies, intermingled with the host nuclei and imbedded in the cytoplasm.



TEXT FIG. 9. A longitudinal section of a hypertrophied cell of *C. contraria* with 18 naked bodies lying in the primordial utricle. The host nuclei are numbered consecutively.

The most striking effect of the parasite, as noted, is the enormous hypertrophy and distortion of the host cell. In this respect it is in line with the symptoms caused by various parasitic slime moulds and chytrids, such a *Plasmodiophora* on the roots of crucifers, *Sorosphaera* on *Veronica*, *Olpidiopsis* on *Saprolegnia* and *Aphanomyces*, *Pleotrachelus* on *Pilobolus* (Zopf, 1888), *Rhizidiomyces* on *Achlya* (Zopf, 1884), *Pseudolpidium* on *Pythium* (Butler, 1907), etc. As the cells swell, the plastids become separated and widely scattered. In addition to causing hypertrophy the parasite appears to stimulate the production of storage starch in the plastids. The plastids of normal cells are generally filled with small lens-shaped autochthonous starch grains during the day, but in cells which have become parasitized the outermost layer of the primordial utricle in which the plastids lie is filled with large round, oval, oblong and irregular storage starch grains as is shown in text figures 7, 8, and 9. The formation of such storage starch is quite common in old dormant *Chara* and *Nitella* cells which persist through the winter, but is not so in actively growing cells. In the final stages of the disease the old hypertrophied *Chara* cells become almost depleted of cytoplasmic content and nuclei.

DISCUSSION

In view of the many similarities in appearance which this organism has in common with the chytrids and parasitic slime moulds it is perhaps pre-

mature to give it a name or assign it to any known family or genus until its complete life history has been worked out. It is by no means certain that it belongs among the chytrids, but the sporangesori of this organism suggest a relationship with the Synchytriaceae, particularly *Woronina*, whose sporangia are grouped into sori. They suggest also in some degree, as noted before, the spore balls of the parasitic slime moulds, such as *Spongospora*, *Sorosphaera*, *Sorodiscus*, and *Ligniera*; but this similarity, it seems to me, is superficial in that the units which make up the spore balls are uninucleated spores rather than sporangia. However, Nemec suggests that these spores are unispored sporangia. In *Sorolpidium*, a genus which Nemec (1911) places next to *Rhizomyxa* in the Synchytriaceae and Stevens (1921) includes among the slime moulds, the sporangia may be uninucleated and give rise to a single spore as in the Plasmodiophoraceae, or multinucleated and undergo cleavage as in the Synchytriaceae. It is to be noted in this connection that the sporangium-like cells which compose the sporangesori of the organism in *Chara* may apparently likewise be uni- or multinucleated. Gäumann (1926) groups the Plasmodiophoraceae and Synchytriaceae together under the Archimycetes, as derived from the Mycetozoa. However, it is premature, it seems to me, to attempt to derive the Synchytriaceae phylogenetically from the slime moulds. The similarities in appearance and structure between the sporangesori of the Synchytriaceae and the spore balls of the parasitic slime moulds may be due to parallelism in development in the two groups.

The presence of caps or opercula on spores and sporangia is characteristic of families as widely different as the Plasmodiophoraceae, Rhizidiaceae, Hypochytriaceae, Cladochytriaceae, and Oochytriaceae. In light of this fact, while the caps or opercula on the cells which compose the sporangesori are somewhat similar to the collars or opercula on the spores of *Sorodiscus* and the sporangia of *Physoderma* and *Cladochytrium*, it is questionable whether they are indicative of family or generic relationship.

I feel grateful to Professor R. A. Harper for the interest he has taken in this study and the generous criticisms which he has so kindly given.

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EXPLANATION OF PLATE XXXII

All photographs were made from living material with Zeiss 16, 8, and 2 mm. apochromatic objectives and compensating oculars Nos. 6 and 8.

FIG. 1. Terminal portion of a leaf of *C. contraria* showing five swollen corticating cells on the internodes.

FIG. 2. An internode of the stem of *C. contraria* with three hypertrophied cells.

FIG. 3. Portion of an internode of the stem of *C. delicatula* with one elongated swollen corticating cell.

FIG. 4. Short hypertrophied corticating cells of *C. delicatula* which were removed from the plant and kept alive for ten days in a culture of tap water.

FIG. 5. Portion of a swollen cell of *C. contraria* showing the scattered appearance of the plastids.

FIG. 6. An old cell of *C. delicatula* with approximately forty sporangesori.

FIG. 7. A small naked multinucleated body.

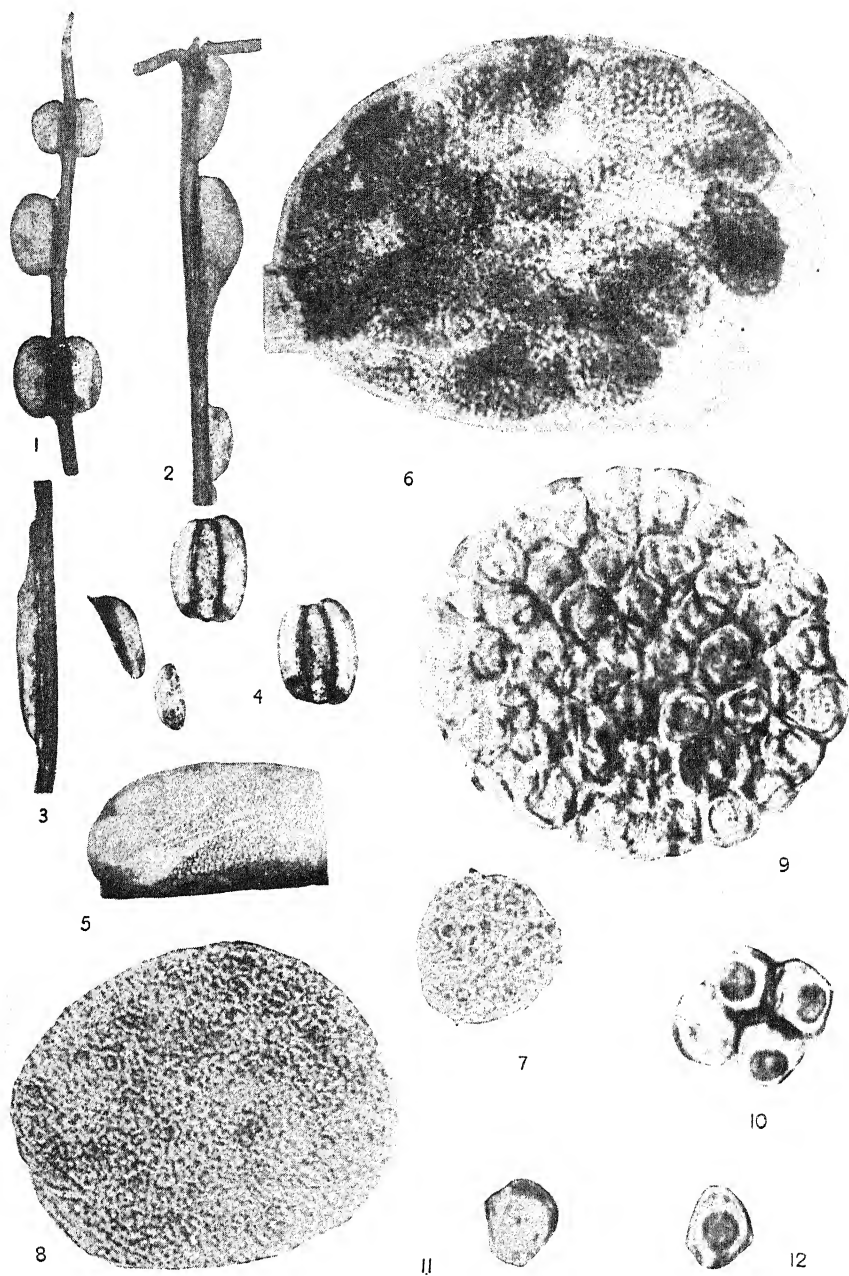
FIG. 8. The same type of body in a later stage of development.

FIG. 9. A single "sporangiosorus."

FIG. 10. Four sporangium-like cells as seen from above.

FIG. 11. A single sporangium-like cell seen in side view.

FIG. 12. The same cell as seen from above.



KARLING: PARASITIC CHYTRID

INTERNAL DECLINE (ENDOXEROSIS¹) OF LEMONS V. CONCERNING THE COMPARATIVE RATES OF WATER CONDUCTION IN TWIGS AND FRUITS²

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INTRODUCTION

This paper presents the results of a continuation of the studies reported in previous papers on the physiological malady known as internal decline or endoxerosis of lemon fruits (1, 2, 3, 4).

Endoxerosis is mainly characterized by a loss of water from the internal tissues of the fruit (1, 2). However, in a very large percentage of instances one of the first visible internal evidences of injury is the presence of gum in the conducting vessels and surrounding tissues, especially in the albedo (white, inner portion) of the peel. When the abnormal physiological conditions persist and become more aggravated, gum becomes more abundant and may be found not only in the peel but in the stylar end of the central axis and may even extend from the inner portion of the peel into the adjoining pulp tissues. A more complete description of this malady may be found in the first paper of this series (1).

The production of gum—pentoses and pentosans (4)—in the fruit materially affects the water-holding capacity of the tissues concerned. Whether in endoxerosis it may also affect the water equilibrium in the tissues of fruit or twig in a mechanical way is to be discussed in this paper and in paper VI of this series, which will appear in the succeeding number of this journal.

AUXOGRAPH RECORDS

During the course of the study on endoxerosis of lemon fruits, auxographs were attached to fruits while in their normal position on the branches to determine the rates and times at which water entered or was withdrawn from the fruits under varying conditions. The shrinkage of the fruits usually indicated that leaf transpiration began to draw water from them at about 6 to 8 A.M. They showed the effect of a decrease in rate of leaf transpiration by beginning to expand again at about 4 to 6 P.M., the actual time of initial expansion and contraction being influenced, of course, by

¹ The term "endoxerosis," as previously suggested (4), is a technical name for this malady, and while it is so used in the text of this paper the term *internal decline* is retained in the title to facilitate reference to this series of papers.

² Paper No. 199, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

climatic conditions. For a more detailed discussion of the use of the auxograph in this connection, see a previous paper (3).

While checking over the auxographic records made during a certain series of tests it was noted that apparently the endoxerotic fruits did not begin to shrink as early in the morning and did not begin to expand as early in the evening as healthy fruits. A special series of tests was then run to obtain further evidence on this point. The results of these tests showed that on an average the endoxerotic fruits began to shrink at 8:15 A.M. and to expand at 5:15 P.M., while the normal fruits on an average began to shrink at 6:50 A.M. and to expand at 4:05 P.M. In other words the endoxerotic fruits began to shrink 1 hour and 25 minutes later and to expand 1 hour and 10 minutes later than the healthy ones on the same trees. The fact that the endoxerotic fruits did not begin to lose water as soon as did the healthy ones might seem on first thought to indicate that the comparatively high pentosan content in the former (4) was the influencing factor, but if this were true the endoxerotic fruits should have begun to expand earlier in the evening than the healthy ones. This did not occur. However, by observing the differences between the average initial times of shrinkage and expansion of the healthy and endoxerotic fruits it will be noticed that the latter is 15 minutes less than the former, and it may be that the water-absorbing power of the pentosans was responsible for this difference.

The results of this series of tests seemed to indicate that some condition within the fruits or in the twigs on which they were borne might be responsible for the differences in times of initial shrinkage and expansion. Further evidence was sought by the use of potometers.

POTOMETER TESTS

Fruit twigs bearing practically mature healthy or endoxerotic fruits were chosen so as to give paired healthy and endoxerotic fruits for these tests. The twigs were borne on twelve trees and were chosen (*a*) at random on the trees, (*b*) where both fruit twigs were on the same large branch, or (*c*) where both healthy and endoxerotic fruits were borne on adjacent pedicels at the end of a fruit twig. The last was true for tests 1, 3, 4, and 11 recorded in table 1. The twigs were allowed to remain attached to the trees but the fruits were severed from the twigs, under water, just back of the button (receptacle and calyx) by means of a sharp razor. The potometers, which were attached to the twigs by means of rubber tubing, were 50 cc. burettes graduated in 1/10 cc. divisions. Distilled water was used in them. The tops of the water columns in the potometers were placed on a level with the cut ends of the twigs so that no hydrostatic pressure would be exerted. The data concerning these tests are recorded in table 1.

TABLE I. *Comparative Volumes of Water Absorbed from Potometers by Fruit Twigs that had Borne Healthy or Endoxerotic Lemon Fruits*

Test Number	Diameter of Lemon (cm.)		Length of Twig (cm.)		Diameter of Twig (cm.)		Water Absorbed (cc.)		Time (Hours)
	H*	E*	H	E	H	E	H	E	
1.....	4.93	5.05	2.54	1.92	0.30	0.33	2.00	1.30	22 $\frac{1}{2}$
2.....	4.97	5.20	5.10	6.33	0.37	0.34	3.80	2.30	23 $\frac{1}{2}$
3.....	5.36	5.30	1.00	1.00	0.32	0.30	1.15	0.70	22
4.....	5.14	5.45	1.20	1.20	0.36	0.37	1.90	0.80	23 $\frac{1}{2}$
5.....	5.13	4.90	4.33	9.71	0.30	0.27	3.90	1.50	21 $\frac{1}{2}$
6.....	5.06	4.77	11.00	5.30	0.28	0.27	1.00	0.85	20 $\frac{1}{2}$
7.....	5.44	5.34	3.09	5.38	0.30	0.28	1.55	1.00	21 $\frac{1}{2}$
8.....	5.64	5.55	7.77	8.53	0.31	0.31	3.00	2.50	22 $\frac{1}{2}$
9.....	5.37	5.30	3.60	2.27	0.30	0.28	1.95	0.90	23 $\frac{1}{2}$
10.....	5.18	5.15	8.27	12.00	0.28	0.27	1.00	1.00	24
11.....	4.73	4.71	0.60	0.60	0.34	0.35	1.50	0.80	22
12.....	5.27	5.20	3.30	3.50	0.32	0.28	3.45	1.70	8 $\frac{1}{2}$
Average	5.19	5.16	4.32	4.40	0.315	0.304	2.18	1.28	

* H = healthy; E = endoxerotic.

The results given in table I show that in every case the amounts of water withdrawn by the twigs that had borne the healthy fruits were equal to or greater than the amounts withdrawn by the twigs that had borne the endoxerotic ones; in fact the total amount withdrawn by the former was almost twice as great as that withdrawn by the latter. As indicated in table I the potometers usually remained attached to the twigs for about 21 to 24 hours. The average amount of water withdrawn by the twigs that had borne normal fruits was 2.18 cc. while that withdrawn by the twigs that had borne endoxerotic fruits was only 1.28 cc. This indicates that some condition in the twig (apparently in the water ducts), and not in the fruit, was largely responsible for the differences in the times of initial shrinkage and expansion, as had been recorded by the auxograph, for healthy and endoxerotic fruits. However, in this statement and in similar ones which may follow it is not meant to imply that some condition in the fruit may not have been at least a partial factor. As indicated in the table there was more or less variation in the sizes of the fruits that had been borne on the twigs and in the lengths and diameters of the twigs, but none of these differences seemed to be the controlling factors. Before making the tests it was thought that possibly the diameter of the twigs bearing the fruits might have an important influence on the results, and by reference to the table it will be seen that the average of the diameters of the twigs which bore the healthy fruits (*H*) was slightly greater than the average for those that had borne the endoxerotic ones (*E*). However, when individual cases are examined it will be seen that this could not have been the deciding factor. For example, in the cases of tests 1, 4, and 11 the twigs that had borne the endoxerotic fruits had the greater diameters, but the

amounts of water drawn through them were much less than the amounts drawn through the corresponding twigs that had borne the healthy ones.

Another example may be cited in which the amounts of water drawn from the potometers were apparently not governed by the diameters of the twigs. In the case of the healthy twigs 4 and 5, the former, with a diameter of 0.36 cm., withdrew only 1.90 cc. of water in $23\frac{1}{2}$ hours while the latter, with a diameter of 0.30 cm., withdrew 3.90 cc. in $21\frac{3}{4}$ hours. Two of the probable factors causing these results were, (a) that growth conditions had produced vessels of a greater conducting capacity in the one case than in the other, and (b) that, as was noted at the time of making the tests, the measurement of the external diameter of the twig did not necessarily record its actual water-conducting capacity because the bark was thicker on some twigs than on others. It should be noted here also that the average relative humidity for the $23\frac{1}{2}$ -hour period during which test 4 was made (1.90 cc. of water withdrawn) was almost 10 percent less than for the $21\frac{3}{4}$ -hour period during which test 5 was made (3.90 cc. of water withdrawn).

That individual variation in the amounts of water drawn from the potometers by the twigs was comparatively great may be shown by a different type of comparison of some of the results shown in table 1. For example, if the amount of water (2.30 cc.) withdrawn by twig 2, that had borne the endoxerotic fruit, is compared with the amount (1.15 cc.) withdrawn by twig 3, that had borne the healthy fruit, it will be seen that the twig that had borne the endoxerotic fruit withdrew more water from the potometer than the one that had borne the healthy fruit. Two or three similar comparisons might be made of the results of other tests. These are directly opposite to the general average shown in the table. The data given in the table, however, seem to justify the conclusion that some condition in the twigs that had borne endoxerotic fruits prevented them from withdrawing as much water from the potometers as did those having borne the healthy ones. The validity of this conclusion is substantiated by the fact that in making the tests the pairs of twigs were always chosen at random, except that the twigs of a given pair were in close proximity, one with the other, on a given branch, and by the fact that in no individual test of paired branches did the twig that had borne the endoxerotic fruit withdraw more water than the one that had borne the healthy fruit. Furthermore, the validity and significance of these results will become more evident when they are linked up with the results shown in table 2.

The detailed records of this group of tests show that approximately 94 percent of the water withdrawn from the potometers was withdrawn during the 14 hours from 5 A.M. to 7 P.M. while only 6 percent of it was withdrawn during the 10 hours from 7 P.M. to 5 A.M.; or, in other words, nearly all of the water was withdrawn during the daylight hours.

The leaves on each twig were counted and the number recorded at the time of making the tests, but these figures have not been recorded in the table

because results previously published (3) indicate that the leaves in question influenced the amount of water withdrawn from the potometers to only a very small extent. The results of the previous test indicate that the amount of water withdrawn from a lemon fruit, and hence the amount withdrawn by a given twig from the potometer, very largely depended upon the water deficit in the tree as a whole rather than upon the transpiration activity of the comparatively small group of leaves attached to the twig bearing the fruit.

FORCING GAS THROUGH SECTIONS OF FRUIT TWIGS

The next step in attacking this problem was to select another set of fruit twigs, similar to those which had been used in the preceding series of tests. In this case, however, instead of having the twigs attached to the trees they were brought to the laboratory where their conducting capacities were tested by forcing carbon dioxid gas through them. The amount of gas passing through an individual twig was measured by attaching its basal end to a cylinder of carbon dioxid, submerging the twig in water, and collecting the emerging bubbles of gas, by the displacement of water, in graduated tubes.³ Centrifuge tubes were found to be well adapted for this purpose because the ends of the tubes taper and small portions of the gas could be fairly accurately measured. In some of the cases, as in the preceding tests, the endoxerotic and healthy fruits were borne on separate twigs while in other cases both kinds of fruits were borne on the forked ends of a single twig, the fork consisting of two adjacent pedicels. Where the two kinds of fruits were borne on separate twigs a glass Y-tube was inserted between them and the gas-tank connection so that the amount of pressure applied simultaneously to each would be the same. The initial tests were made by "pulling" the fruit from the twig, thus leaving the button attached to the twig. Here again, as in the preceding series of tests, the fruits were practically mature and the average of their diameters was approximately the same as for those indicated in table 1.

After one series of gas readings had been taken, the button was cut from the pedicel and a second series of readings was then taken. This process of cutting off successive portions of the pedicels or twigs and taking new readings was repeated until the pedicels had been practically entirely removed in the cases where both kinds of fruits had been borne on the same twig or until three to six pieces had been cut from each of the twigs on which the healthy and endoxerotic fruits had been borne separately. About $\frac{1}{2}$ to 1 cm. of the pedicel or twig was removed at each cutting. This meant that only two to three readings could be made where both kinds of fruits were borne on the same twig. The initial length of the pieces of twigs tested was from 7 to 9 cm. 104 twigs were used in this series of tests, thus entailing almost 400 readings. The bark was not removed from either

³ Tests were also made with nitrogen but no difference in the results with the two gases could be detected.

end of the twig which was attached to the tube from the pressure tank. This point will be discussed at the end of this section of the paper. The amount of pressure applied to all of the twigs was the same, as nearly as could be regulated and determined by means of the needle valve, pressure gauge, and mercury manometer attached to the gas tank. While there was a slight variation in pressure for the different tests it was approximately two thirds of an atmosphere in each case.

The mass of data obtained in these tests is too great to include in its entirety in this paper, and therefore the results of only a few of the tests are given. The cases cited are representative of the entire series and are presented in table 2. It should be noted that, in the first five cases cited in the table, twigs were used which had borne a healthy and an endoxerotic fruit side by side on adjacent pedicels on the end of the same twig. In these cases the twig length as given in the table refers only to the length of the pedicels. Usually the twig was cut about 4 cm. back of the fork formed by the pedicels, and the tube from the pressure tank was attached at that place. In the other five cases cited in the table the healthy and endoxerotic fruits were borne on separate twigs.

The principal points of interest concerning the results recorded in table 2 may be summarized as follows:

1. New evidence was obtained for concluding that some condition in the twig, and not in the fruit itself, was at least principally responsible for the fact that the auxographic records had shown the initial times of shrinkage and expansion for the endoxerotic fruits to be later than those for the healthy ones. This is indicated by the fact that the initial readings of only two of the ten cases cited showed that more gas could be forced through the twigs that had borne endoxerotic fruits than through those that had borne healthy ones (see tests 3 and 23, table 2). It should be noted here that there were only three such cases in the entire series.

2. In most cases the amount of gas that could be forced through the twigs that had borne endoxerotic fruits was markedly less than the amount that could be forced through those that had borne healthy ones. This was found to hold true more especially for the results of the first one to three readings of each comparative test.

3. As successive portions of the pedicels or twigs were removed the comparative amounts of gas that passed through the twigs that had borne the two kinds of fruits (healthy and endoxerotic) became approximately equal, *i.e.*, within the limits of individual variation. In many cases, however, it was of interest to note that although the initial readings showed that the twig that had borne the healthy fruit was emitting more gas than the one having borne the endoxerotic fruit, subsequent or final readings showed the conditions to be reversed. Forty percent of this series showed reversals similar to those illustrated by tests 22 and 39, table 2. In both of these cases the conditions were not reversed until the time of the final

TABLE 2. *Healthy and Endoxerotic Lemon Fruits and Their Twigs Showing Their Sizes and the Comparative Amounts of Gas that Could be Forced Through the Twigs*

Test Number	Diameter of Lemon (cm.)		Length of Twig (cm.)		Diameter of Twig (cm.) †		Volume Gas per Minute (cc.)	
	H *	E *	H	E	H	E	H	E
2a † . . .	4.82	5.00	1.00	1.00			4.00	0.05
b			0.50	0.50	.29	.32	14.80	2.60
4a	5.00	5.44	1.00	1.00			2.85	0.15
b			0.50	0.50	.32	.33	11.40	1.40
6a	5.43	5.17	1.00	1.00			2.75	0.10
b			0.75	0.75	.40	.39	17.00	2.20
7a	5.76	5.78	1.50	1.50			2.10	0.10
b			1.00	1.00	.36	.34	39.60	19.20
c			0.50	0.50	.36	.34	64.80	49.20
3a	4.90	5.54	1.00	1.00			0.67	0.80
b			0.50	0.50	.36	.38	10.20	12.60
11a	5.45	5.70	8.50	8.50			0.63	0.07
b			7.50	7.50	.30	.25	7.50	1.60
c			6.50	6.50	.31	.30	18.20	5.60
d			4.00	4.00	.33	.32	31.60	14.00
e			2.50	2.50	.31	.32	73.20	34.20
22a	5.55	4.75	5.50	5.50			0.93	0.07
b			5.00	5.00	.31	.32	2.80	0.80
c			4.00	4.00	.27	.27	9.10	3.90
d			2.00	2.00	.24	.28	11.20	23.20
23a	5.37	5.53	4.00	4.00			1.85	3.30
b			3.50	3.50	.32	.32	5.60	10.00
c			2.00	2.00	.31	.31	9.60	20.80
39a	5.89	5.87	4.00	4.00			1.80	0.55
b			3.50	3.50	.32	.37	4.60	4.00
c			2.50	2.50	.33	.41	16.80	14.80
d			1.50	1.50	.28	.31	21.60	39.00
52a	5.35	5.42	6.95	6.95			1.80	0.00
b			6.54	6.60	.35	.35	5.00	0.40
c			5.46	5.55	.34	.34	10.60	0.80
d			4.42	4.48	.32	.34	21.90	2.10
e			3.52	3.32	.35	.38	28.80	12.00
f			2.52	2.32	.35	.40	49.80	24.00
g			1.63	1.37	.36	.42	190.00	110.00

* H = healthy; E = endoxerotic.

† It will be noted in the table that in each case no initial diameter measurement is given for the twig. This indicates that the twig still retained the button and no attempt was made to measure the diameter of the buttons.

‡ The a, b, c, etc., of each number denote successive measurements as each new portion of the twig was cut off.

reading, while in some of the cases not reported the reversal came as early as the second or third reading. This would seem to indicate that aside from the first few centimeters at the apical end the two sets of twigs, though showing individual variation, had a similar gas-conducting capacity.

4. There was considerable variation in the amounts of gas that could be forced through healthy twigs of approximately equal lengths and diameters, just as there had been a variation in the amounts of water that similar twigs withdrew from the potometers. For example, the amounts of gas that could be forced through three consecutive sections of healthy twig No. 12 (not given in the table) were 5.00, 16.60, and 32.40 cc. per minute, respectively, while healthy twig No. 23 passed only 1.85, 5.60, and 9.60 cc. of gas per minute. The initial lengths of these twigs and the successive amounts removed from each twig following a series of readings were equal.

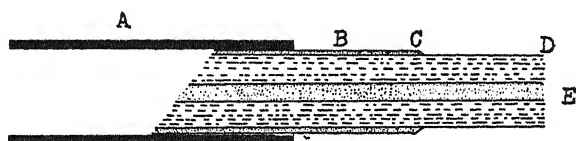
The comparatively great variations in the amounts of gas that could be forced even through healthy twigs was not wholly governed by their length or diameter. Since it seemed probable that only those water-conducting vessels whose open ends were exposed at each extremity of the twig could conduct the gas, it was thought well to determine in at least a general way the distances between the gas-impermeable cross walls in vessels of lemon twigs of the age that had been used in making the gas tests. The lemon twigs chosen were approximately one year old, but some of them had grown slowly while others had grown rapidly. The tests were made by using a 40-cm. head of mercury, a 70-cm. head of a 0.20-percent starch solution, or a gas pressure of one atmosphere. The results of the tests by all of these methods were very closely similar; in fact, in some cases they were identical. The basal ends of the twigs were attached and then successive short portions of the apical ends were cut off until the test substance being used began to emerge. By using a magnifying glass it was possible to determine for a time the number and the location of the points of exit, *i.e.*, the number of vessels that were open the entire length of the twig.

The results of this test indicated that the lengths of the open segments⁴ of the vessels in lemon twigs one year old may range all the way from 10 to 40 cm., the average being nearer the lower figure. The longest open vessel segments were found in the rapidly growing twigs. In this connection it may be of interest to note that apparently the older the twig the longer the open segments in the vessels, *i.e.*, as the twig becomes older more cross walls in the vessels disintegrate, thus lengthening the distance between cross walls that are still intact. As proof that this is true it may be stated that in lemon twigs which were two years old the open vessel segments were found to be at least 72 cm. long (the maximum found in twigs one year

⁴The term "vessel segment" is used in this paper to designate the open portion of the water-conducting vessel between two cross walls which acted as barriers to the passage of gas or other test substance used.

old was 40 cm.), while in twigs three years old the segments were found to be at least 102 cm. long.

In the previous tests where gas was used the closed cross walls in the vessels appeared to prevent completely the passage of gas under the pressure used, but at this point the questions might well be raised (*a*) as to whether all of the gas that was forced through the twigs in the different tests where gas was used actually passed through the vessels, or whether at least part was forced through the parenchyma in the pith and bark, and (*b*) as to whether the gas which passed through the xylem traveled mostly in the older and less active vessels near the pith, through those near the cambium, or about equally through the vessels in all portions of the xylem.



TEXT FIG. 1. The method of determining whether gas could be forced longitudinally through the bark of a twig. A, rubber tubing from gas tank; B, bark; C, edge of bark after being peeled back and cut away; D, xylem, and E, pith.

The answer to the first question was obtained in the following manner: Twigs approximately one year old were used; in this case, however, they were only 5 instead of 7 to 9 cm. long, since if gas could be forced through the pith or bark it could be done more easily through a short twig than through a long one. In this case, too, the pressure used was greater, being 1.14 atmospheres instead of two-thirds of an atmosphere as in the preceding series of tests. The tube leading from the pressure tank was slipped on over one end of the twig without removing the bark, the gas from the pressure tank turned on, and from three to four successive measurements of the gas-conducting capacity of the twig were made. Then while allowing the gas to continue to flow through the twig under the same pressure, from 1 to 2 cm. of the bark was peeled back, with the aid of the fingers, from the distal end of the twig. These strips of bark were then cut off as close to the stem as possible with a razor, the cut being made each time away from the stem (see text fig. 1). The fingers were used in peeling the bark back to avoid making an abrasion in the newly exposed surface of the xylem. After the bark had thus been removed the twig was again submerged in water and a second series of three to four measurements was made of the rate at which gas was being emitted from the bark-free end of the twig (at D, text fig. 1). The rate for each of the lemon twigs tested was the same as before the bark had been removed. This condition could mean but one thing, namely, that no appreciable amount of gas passed through the bark. Had gas been doing so, it could have been easily seen, in the second half of the test, arising in the form of bubbles from the cut

margin of the bark (at C, text figure 1) which in this case was 1 to 2 cm. back of the distal end of the xylem. In a few cases an occasional minute bubble could be seen coming from the cut end of the bark at C, but it would have taken hours to obtain a measurable amount of gas. This amount would be negligible compared with the 5 to 35 cc. per minute that passed through the xylem.

It may be of interest to state here that the same kind of a test was made on twigs of English walnut, orange, Japanese elm, *Eucalyptus*, and *Pittosporum*. The pieces of these twigs were $5\frac{1}{2}$ cm. long. They were all just a little over one year old except the orange, which was one cycle of growth over two years old (*Citrus* usually makes three cycles of growth during each growing season), and the Japanese elm, which was a little over four years old. The results with the walnut, *Eucalyptus*, and *Pittosporum* were the same as with the lemon twigs, i.e., no appreciable amount of gas passed through the bark. In the case of the two-year-old orange and the four-year-old Japanese elm, 0.15 cc. and 1.50 cc. of gas per minute, respectively, passed through the bark, emerging at C, text figure 1. These amounts may appear rather large, but comparatively they are not, since while the 0.15 cc. of gas was being emitted from the bark of the orange twig (at C), 32.5 cc. escaped from the end of the xylem from which the bark had been removed (at D); and while the amount of gas that came from the bark of the Japanese elm (at C) was 1.50 cc. per minute, the amount that came from the xylem (at D) was 696 cc. during the same period.

In no case was there any indication that gas was escaping through the surface of the bark or through the freshly exposed surface of the xylem after the bark had been removed.

By making a long slanting cut, by reducing the gas pressure so that the bubbles caused by the gas coming from the vessels of the xylem did not obscure the view, and by using a magnifying glass, it was determined that gas did not pass through the pith of any of the twigs tested, at least not while the reduced gas pressure was being used. From this it does not seem probable that a consequential amount, if any, passed through it under the pressure used in making the previous tests.

The question as to whether the gas was being emitted from any particular portion of the cross-sectional area of the xylem more than from another was determined by again making a long slanting cut from the free end of the twig and by observing the places of origin of the gas bubbles with the aid of a magnifying glass. As nearly as could be detected the gas appeared to be coming equally from all portions of the xylem. Although gas was escaping from what appeared to be a large number of vessels, in reality it came from only a few compared with the total number of vessels exposed on the cut surface of the xylem. Apparently it could pass through and escape only from those vessels whose open segments were exposed at both extremities of the twig.

That gas was passing through the newly formed vessels adjoining the cambium was proved in still another manner. The twig was attached to the pressure tube as before, the bark peeled back from the distal end, the twig submerged in water, the pressure turned on, and a very slight needle scratch made transversely across the freshly exposed xylem, anywhere between *C* and *D*, text figure 1. Bubbles began to arise at once from the surface of the xylem where it had been scratched by the needle. The deeper the scratch the greater the number of bubbles, but even the very slightest scratch would allow gas to escape. This simple test indicated very plainly that even the most newly formed vessels were contributing their share to the amount of gas escaping at the end of the twig.

CONCLUSION AND SUMMARY

1. Auxographic records, recording fluctuations in rates and times of withdrawal of water from and its entrance into lemon fruits, as influenced by diurnal factors, had shown during previous tests that endoxerotic fruits began to shrink 1 hour and 25 minutes later and to expand 1 hour and 10 minutes later than healthy ones. This indicated that the inherent water-conducting capacities of the two sets of twigs were different, or that some condition in the endoxerotic fruits, or in the twigs on which they were borne, was acting as a barrier to the normal rate of water conduction.

2. By removing both healthy and endoxerotic lemon fruits from their pedicels and attaching potometers to the ends of these twigs while they were attached to the trees in their normal positions, it was found that some condition in the twigs that had borne endoxerotic fruits, and not in the fruits themselves, was at least principally responsible for the retardation in the rate of water conduction. The twigs that had borne the healthy fruits withdrew almost twice as much water from the potometers as was withdrawn by those that had borne endoxerotic fruits.

3. By bringing similar twigs to the laboratory and comparing the amounts of gas that could be forced through them, further evidence was obtained that some condition in the twigs that had borne endoxerotic fruits prevented them from conducting water as rapidly as did those that had borne healthy ones. Just as the twigs that had borne healthy fruits withdrew larger amounts of water from the potometers, they also conducted larger amounts of gas until the distal portion of the twigs had been removed to a point some 2 to 5 or 6 cm. back of the fruit, after which the rates of conduction were approximately the same, *i.e.*, within the limits of individual variation.

4. A somewhat limited number of tests indicated (*a*) that the open segments of the vessels in the xylem of lemon twigs one year old range from 10 to 40 cm. in length, (*b*) that as the xylem becomes older more cross walls disintegrate, thus producing open vessel segments of greater length, and (*c*) that the cross walls in the vessels act as barriers to the passage of gas,

making the amount of gas conducted by a given piece of twig proportional to the number of open vessel segments exposed at both ends of that particular twig portion.

5. A special series of tests was made on healthy twigs which showed (a) that the gas which was being forced through the twigs passed through the vessels of the xylem and not through the intercellular spaces in the parenchyma of the bark or the pith, and (b) that all portions of the xylem, both young and old, appeared to be equally active in conducting the gas.

6. Experimental evidence did not indicate that a difference in the number of vessels, in the lengths of their open segments, or in their cross-sectional area, could be responsible for the fact that the twigs that had borne healthy fruits conducted more water and gas than those that had borne endoxerotic fruits, nor did microscopical examination reveal any tyloses in the vessels of either set of twigs.

Just what condition in the portion of the twig immediately back of the fruit, and in the fruit (if any), was responsible for these differences will be discussed in paper VI of this series, which will appear in the succeeding issue of this journal.

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HISTOGENESIS OF INTUMESCENCES IN THE APPLE INDUCED BY ETHYLENE GAS

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The development of pathological outgrowths or intumescences in the roots, stems, and leaves of many species of plants is known to involve fundamental tissue modifications. These modifications usually consist of a more or less complete disorganization of the tissue masses by hypertrophy or hyperplasia or both, and may in extreme cases result in the complete separation of the tissues and the liberation of their individual protoplasts. Such abnormalities may arise in response to a wide variety of stimuli. Excessive temperature or moisture and other abnormal environmental conditions commonly induce them in many plants. Likewise, stimulation by chemicals or by fungus organisms frequently leads to their formation. Even the physical stimulation of friction has been known to cause them. I have described in two other papers (1926, 1927) the gross morphological characteristics of intumescences which develop in the buds and stems of apple in response to chemical stimulation by ethylene gas, and I have also reported experiments showing the effect of various external and internal conditions on the production of these outgrowths.

REVIEW OF LITERATURE

Abnormal outgrowths similar in general external characteristics to those developing in the tissues of the apple in response to ethylene gas are known to form in the tissues of many woody plants, as I have noted in my previous papers. They may be relatively superficial, involving only the phellogen and the outer layers of the cortex, or deep-seated cankers penetrating to the cambium, depending upon the experimental materials and methods used.

Histological and cytological data relative to the changes which occur in the various tissue and cellular structures during the formation of these abnormalities are very fragmentary. Hypertrophy of cells is usually found to be the most conspicuous change which occurs in the tissues. Sorauer

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(1908) described the dropsy disease of *Ribes aureum*, an example of the more extreme type of bark overgrowth, as consisting of a more or less complete disorganization of the tissues through hypertrophy of the cells. The hypertrophy may reach to and involve the cambium in the extreme cases, but more commonly only the cells of the more superficial tissues are enlarged. The cells of the bark elongate radially to form a spongy, soft, callus-like tissue mass, which pushes out and ruptures the corky layers. The unmodified bast strands are still present in the mature intumescence and are very often arched outward owing to the pressure of the swollen tissues beneath. Cell counts which he made on the healthy and diseased tissues showed only an insignificant increase in the number of cells in the diseased areas. This substantiates his conclusion that hyperplasia constitutes only a very minor element in the development of these swellings. Sorauer gave no definite data as to the nature of the chemical or structural alterations in the cell walls which might make possible the enlargement and liberation of the cells.

Shilling (1915) made a more detailed histological study of the intumescences which can be induced in the bark of various woody stems by coating them with an impervious layer. For *Clerodendron* and some other genera he concluded, on the basis of the staining reactions, that a softening of the walls preceded the enlargement of the cells. This alteration in the chemical composition of the walls allowed the turgid protoplast to enlarge and stretch the walls, thus forming the thin-walled, hypertrophied cells of the intumescences. He gave no data as to changes in the middle lamella.

The most complete account of the changes in the walls during the separation of the cells in a complex tissue is that of Lloyd (1916). In the processes which led to the formation of the abscission layer in *Mirabilis jalapa*, Lloyd found that the first visible change was a chemical alteration of the radial walls of the cells of the future separation layer. The changed walls stained differentially with Bismarck brown, ruthenium red, and other dyes. Ultimately there is a more or less complete solution of the primary and secondary walls, as indicated by their gradual loss of staining capacity. The protoplasts then enlarge greatly and stretch the tertiary cell membranes so thin that they can scarcely be resolved by the highest magnifications. These attenuated membranes continue to invest the protoplasts when the cells become free. The fact that the walls have been digested very irregularly is strikingly evident in Lloyd's figures. Portions of apparently unaltered wall are often suspended between other portions of which only the extremely thin tertiary membranes remain. His figure 1, *e* indicates strongly that this dissolution of the primary and secondary wall layers may be accompanied by only a slight increase in the size of the cell. He concluded that the secondary thickenings and middle lamella are probably digested away simultaneously.

MATERIALS AND METHODS

The plant materials used in this study were buds and stems of *Pyrus Malus*, Var. "Transparent,"¹ treated with ethylene gas as described in my other papers (1926, 1927). Branches from 1 to 1.5 cm. in diameter at the base were taken from trees growing out-of-doors. These shoots were cut into short lengths (10 to 15 cm. long) and placed in bell-jars. Ethylene sufficient to make up one percent of the atmosphere was then liberated in the jars.

The corky layer was removed from the old abscission scar at the base of the buds, as described in my other experiments (1927), thus insuring responses in almost every bud. Materials were removed from the bell-jars and killed and fixed at intervals of four hours through a period of four days. At the end of this time the intumescence response in the buds is complete. A similar series killed and fixed at intervals of twenty-four hours after exposure to the gas was used for the study of the intumescences developing at the cut ends of twigs.

The present study was restricted to the bark between the phellogen and the true cambium and to such portions of young xylem as were present in the bud-bases. Mature xylem rarely if ever reacts to ethylene. Materials for the study of the buds consisted of the bud itself, the bud-base, and enough of the surrounding bark to enable me to orient the bud on the stem. Materials for the study of the intumescences in the cut ends of twigs and in the internodes were simply longitudinal segments of the bark through both normal and swollen regions.

Nèmec's chrom-formalin killing solution was used for most of the preparations. I found from several tests that Flemming's, Merkel's, Nawaschin's, and several other solutions failed to give as satisfactory results. Nèmec's solution gave good killing and fixing with a minimum of plasmolysis and shrinkage, and, moreover, had no apparent hardening effect on the bast fibers. It was possible to obtain very good sections of the material 7.5 microns thick. Also, the material cut better when chloroform was used as the paraffin solvent. Flemming's triple stain was used throughout.

RESULTS

The response exhibited by the buds and the stems of apple when stimulated by ethylene gas consists of a disorganization of the tissues, leading ultimately to the formation of swollen, functionless cankers, which I have previously described as intumescences. These lesions arise through the solution of the walls, and hypertrophy and proliferation of the cells.

It has been emphasized in my previous papers (1926, 1927) that these

¹ Studies made by the author on three varieties of apple indicate that there is considerable varietal variation in the capacity to form intumescences. "Transparent" has always shown the greatest capacity for such a response. The others tested form intumescences weakly or not at all.

outgrowths may develop in the buds, and in the proximal and distal ends and in the internodal regions of stem cuttings of the "Transparent" apple. I have also shown that the structural features and times of development of the swellings differ somewhat for the three regions. I have since determined that the fundamental changes in the tissues are the same in the different regions, varying only in the time of development and in the completeness with which the cells are set free. The following descriptions apply to intumescences in all three regions unless it is specifically stated to the contrary.

Solution of Walls

The most characteristic change in the tissues during the formation of the outgrowths is the dissolution or digestion of the cell walls. This results in the more or less complete separation of the cells from tissue continuity, and the rounding up of the individual protoplasts.

The destruction of the walls consists chiefly in the solution of the secondary thickening. This proceeds very irregularly. Certain restricted areas completely disappear before adjacent portions of the same wall become appreciably modified. The appearance is that of an unequal corrosion, which is most characteristically shown in the walls of the collenchyma. The unmodified secondary walls of this tissue stain an intense yellow-red with Flemming's triple stain. Slightly changed regions of the wall take the stain with a greatly reduced intensity (*b*, figs. 1, 4, Pl. XXXIII). The staining capacity continues to decrease, and finally just prior to complete dissolution it is so slight that with ordinary treatment the wall remains colorless (*c*, fig. 4). These corrosion figures in the apple tissue are almost identical with those described by Lloyd (1916) for the walls of abscission cells of *Mirabilis*.

The middle lamella seems to have been destroyed just before or at the time of the final dissolution of the secondary wall thickenings. The middle lamella stains red-brown very readily in normal walls, but it is difficult to demonstrate in the modified ones. The exact time of its disappearance is, however, hard to determine. There can be no doubt that it is still present in an apparently unaltered condition in portions of walls adjacent to areas which have undergone complete solution (figs. 1, 2), and it is equally clear that it is absent or too greatly modified to stain when the secondary wall has lost its staining capacity (*c*, fig. 4).

There is some evidence that swelling may occur during any stage of the corrosion process, but it is not sufficient to justify the conclusion that it must always accompany the dissolution of the walls. Very often a slightly altered portion of a wall, as shown at *a* in figure 4, may show distinct swelling, while similar portions of the wall in the same or neighboring cells may show no apparent enlargement. These conditions seem to hold true for the other stages of the corrosion process, as can be seen in figure 4.

A single section (fig. 4) through a rather well developed intumescence

may show cells in all stages of wall dissolution. The cells in such a section may vary from those which are apparently unaffected to those which have become entirely free from tissue continuity through the complete destruction of their heavy walls. Each protoplast when free apparently remains invested by an extremely delicate tertiary membrane. These enclosing membranes are easily demonstrated by plasmolytic experiments. Lloyd (1916) observed such membranes around the abscission cells of *Mirabilis*. He concluded from dissection experiments that they were present originally lining the cell lumen and had simply become free with the cell when the rest of the wall had been hydrolyzed away.

All living elements between the phellogen and the true cambium may undergo this corrosion of the walls and the liberation of the cells from the tissue masses. Figure 6 shows a cross section of a one-year-old stem of cultivated crab with a well developed intumescence. The plant failed to form a protective cork layer, so that the affected and unaffected tissues merge into each other.

In general the parenchyma is most affected, but even the bast fibers may completely disappear in the diseased regions. At the left in figure 6 there are four or five crescent-shaped groups of unmodified bast fibers, but toward the right of the section they become altered and finally disappear altogether. The deep-staining tannin cells which surrounded these groups are still visible near the base of the intumescence. The walls of the young xylem (from bud-bases) may likewise be dissolved out. Water mounts of free cells from a bud intumescence may also show the spiral thickenings of these vessels floating entirely free of their walls. A more detailed study of the breaking down of the walls of these supposedly non-living elements is needed.

Orthorhombic calcium oxalate crystals are often very abundant in the disorganized tissues of an intumescence. These crystals are frequently found arranged end-to-end in long rows rather than scattered through the tissues. A possible explanation of this tandem arrangement of the crystals becomes evident from a study of the corrosion of the bast fibers. Longitudinal and cross sections of the bark containing bast-fiber groups in various stages of dissolution show definitely that these crystals may form in the lumen of the bast fibers while the walls are undergoing solution. They are very small when they first appear inside the fibers, but continue to grow as the dissolution of the wall progresses, and may finally exceed in size the diameter of the original fiber itself. Numerous rows of these crystals arranged as noted are often found still roughly outlining the position of the original bast fiber groups. The abundance of calcium oxalate as a possible by-product of the digestion of the walls may throw some light on the enzymatic process which is responsible for the destruction of the walls.

In the early stages of the development of the intumescences very definite corrosion zones are usually found ramifying through the tissues. Between these zones or regions lie tissue masses apparently unaffected by the gas.

The general character and distribution of these irregular zones is shown in figure 9. The dotted lines in figure 4 show how definite these zones may be with respect to the individual cells. Every cell in this figure showing corrosion of its walls is a part of at least one of the three main corrosion zones. Very often two or more of the zones may intersect (as near *b, b*), and a more extensive area involving several cells results.

These zones usually run more or less parallel with the long axis of the stem. This is evident in the base of the intumescence in figure 4, and is very conspicuous in the body of the swelling shown in figure 9. Even in figure 9, which shows only about one-tenth of an intumescence, several zones are fifty cells long. It is interesting to note, in view of the probably limiting effect of the corky layer, described below, that the most extensive zone in figure 4 ends in a cell adjacent to the cork layer itself. The evidence clearly indicates that these zones of corrosion are diffusion tracts for the ethylene or the stimulus which it initiates.

Cell Hypertrophy

There is an enormous increase in the size of most of the cells which make up the outgrowths. This hypertrophy, as in other plant oedemas, accounts for most of the swelling of the affected tissues.

Enlargement of the cells, however, does not seem to be necessarily the primary cause of the freeing of the cells from tissue continuity. Very frequently a cell will show complete corrosion of its walls in certain regions, with no obvious enlargement. The single cell in figures 1 and 2 shows this condition. Certain portions of the wall of this cell have been completely corroded away, other portions have been greatly modified, while still others are apparently unaltered. Obviously the protoplast has enlarged very little, if any, since it does not protrude into the spaces (*d*) left vacant by the disappearance of the wall material. Several cells in figure 4 show a similar condition. Lloyd's figure (1, *e*, page 220, 1916) strongly suggests that the same may be true for the abscission cells of *Mirabilis*.

Enlargement of cells, however, often occurs simultaneously with the break-up of the tissue masses. This is shown in figure 4, where many of the cells whose walls are undergoing modification have swollen greatly. There is little or no evidence that the turgor of the protoplast stretches the modified walls. If this were true, the altered walls should thin out in a manner similar to that of an elastic band under tension. Walls showing this effect are relatively rare, except in the phellogen, as mentioned below. Usually the corroded regions of a wall end abruptly as shown in figure 1. An examination of figure 4 will show that this abruptness is conspicuous even when the walls are drawn to the small scale of this figure.

The size of cells in the tissues of an intumescence may vary within wide limits. Measurements made of cells just after they were set free from the tissues at the base of old leaf abscission layers indicate that there may be

only a very slight increase in size prior to this time. This might easily be possible if digestion of the walls alone were able to free the cells. In table I are given measurements of several hundred cells taken both before and after the breaking up of the tissues. The average diameter (both length and width) of four hundred unaltered parenchyma cells lying just below old leaf abscission layers at the base of buds was twenty-seven microns. A similar group of two hundred cells from the same position, measured shortly after the break up of the tissue mass, gave an average of twenty-nine microns.

TABLE I. *Size of Cells in Normal and Intumescence Tissue of "Transparent" Apple*

Number of Cells	Average Size of Cortical Cells from Base of Old Leaf Abscission Layer				Average Size of Cells from Mature Intumescence	
	Normal Tissue		Free Cells *		Length	Width
	Length	Width	Length	Width		
100	30.76 μ	23.84 μ				
100	25.19	22.10				
100	26.87	26.15				
100	32.94	30.32				
200			29.12 μ	29.05 μ		
115 †					56.00 μ	44.16 μ
15 §					279.20	54.08
Ave. . . .	28.99 μ	25.60 μ	29.12 μ	29.05 μ		

* The intumescences from which these cells were taken were very young. The position of the cells showed that they had just been set free from tissue continuity.

† This group of cells may be taken as representing the average size of cells in the intumescences. This average includes cells that varied from those which were extremely large to those which were very small.

§ These few cells were selected from the previous groups as representing the very largest cells present. They did not constitute more than one percent of the cells present in the preparations.

The marked cell enlargement of later stages seems to follow the partial freeing of the cells. The presence of ramifying zones or areas of greatly enlarged cells in figure 9 supports this conclusion. In table I are measurements of cells in water mounts of free cell units from a mature intumescence. The average diameter of these cells is about twice that of normal cortical cells. The average size of fifteen of the largest cells present in a water mount of living material was 279×54 microns. The largest single cell of this group was 365×58 microns, which is well within the range of vision of the unaided eye. These giant cells were relatively rare, however, constituting only one percent or less of the total number of cells in the mounts. The giant cells differ from the typical cells of the intumescences in shape, being much elongated rather than oval.

All the living cells of the bark may undergo this hypertrophy. Not uncommonly the ray cells show a more pronounced enlargement than other cells of the phloem. The sieve tubes do not enlarge appreciably, and may often be found in young intumescences in an apparently unaltered condition free among the other cells.

The portion of the bark which undergoes the most pronounced cell hypertrophy or enlargement is usually the cork cambium. The true cambium, on the other hand, shows no special tendency to enlarge. The wide-spread, superficial, blister-like intumescences which I have described (1927) are due to hypertrophy of the phellogen. The cells enlarge radially, forming a palisade-like layer in which they are frequently ten times as long as they are wide (fig. 5). This radial distention of the protoplast apparently stretches the radial walls. I have been unable to find definite wall corrosion figures in the phellogen such as I have described for the other tissues of the bark. There is usually a gradual decrease in the thickness of the wall as one passes from normal to elongated cells. This transition can be seen in figure 3, which also shows that the tangential walls, at least the inner one, may remain apparently normal. The meristematic condition of the phellogen may possibly be the cause for this variation from that which is typical for the other regions.

With sufficient care one can remove the hypertrophied phellogen as a sheet of friable tissue. Microscopic examination of a water mount of a sheet of this tissue might easily lead one to conclude that the cells were spherical, since they are then seen endwise. A slight tapping on the cover glass, however, will cause the friable tissue to break up, and the long palisade-like cells will float free. In this modification of the phellogen there is no evidence that any great number of the cells have undergone division.

Hyperplasia

Proliferation of the cells of any living tissue of the bark may occur during the formation of these intumescences. These cell divisions in the intumescence proper seem to bear no relation to the localized divisions which result in the formation of the protective cork layer. They are scattered at random between the phellogen and the true cambium, and show no special grouping or zoning with respect to any tissue or position in the stem. The division figures were normal throughout, with no evidence of irregularities either in the chromatic or achromatic elements. Only rarely do two or more adjacent cells undergo division simultaneously. In a few instances cells which had begun to hypertrophy divided, but in most cases the dividing cells were apparently unaffected by the gas.

The percentage of increase in the cell number accompanying the formation of intumescences in the end of cuttings was determined to be about 33 percent. This value was computed from a series of parallel counts of cells present between the phellogen and the true cambium in normal and

TABLE 2. *Number of Cells Present in Radial Sections of Bark Between the Cork Cambium and True Cambium*

Slide No.	Counts	Cells Present		Slide No.	Counts	Cells Present	
		Normal	Swollen			Normal	Swollen
1	1	31	50	12	4	35	40
2	1	36	42			35	44
3	1	30	40			40	45
4	1	32	47			34	42
5	1	34	48	13	4	36	50
6	1	35	49			38	45
7	1	37	42			37	51
8	3	34	46			38	48
		35	50	14	4	33	54
		34	48			36	52
9	4	31	40			39	51
		29	42			35	52
		30	43	15	4	34	44
		32	40			35	45
10	4	34	42			35	51
		37	42			42	45
		35	43	16	4	34	45
		31	44			38	42
11	4	36	50			31	46
		35	51			31	42
		40	48	17	4	33	52
		35	46			35	49
						35	44
						36	43
Total					46	1588	2115
Average number of cells per section						34.5	46.0

swollen regions of the same sections. The intumescences from which the preparations were made were of the type classified by me as medium (1927), and therefore represent neither very strong nor very weak responses for the material. The cuttings were approximately .5 cm. in diameter and of uniform size, and were made from branches of the current year. In table 2 are presented the data obtained from the cell counts on this material. It will be observed that there is little variation in the number of cells present in radial sections of the bark of these cuttings. Mathematical computations based on the above data give $34.5 \pm .36$ as the average number of cells in a section of the normal region, and $46.0 \pm .57$ as the average for the swollen region. This difference of 12 in the average number of cells in the two regions gives a calculated increase of about 33 percent in the number of cells for the affected tissue.

Mature Condition of Intumescences

Only the cork cells remain in tissue continuity when the intumescences in the stems and buds have reached their climax development. The living tissues disintegrate into a friable mass of unspecialized rounded cells which have lost all their normal structural and physiological connections. This

climax stage is common in the buds and bud-bases, but in the case of inter-nodal swellings usually only localized regions of the bark show this complete break-up. More frequently the maximum development which an intumescence in the bark may attain is that shown in figure 9. In an intumescence mass of this type one may find among the living cells various specialized elements such as sieve tubes, bast fibers, spiral thickenings, etc., in various stages of disintegration.

Two forms of calcium oxalate crystals are generally present: the stellate type, usually found in the cortical parenchyma cells near the collenchyma; and the orthorhombic type, usually in the deeper regions, especially in that of the hard bast. The stellate forms are as common in the control as in the ethylene-treated material. The orthorhombic forms, on the contrary, are much more abundant in the treated materials, and may have arisen, as I have noted above, in connection with the disintegration of the bast fibers. These crystals are, however, often found abundantly in regions at some distance from the hard bast.

I have described (1927) the general characteristics of the thin-walled, hypertrophied, free cells of the mature intumescence. The evidence here bears out Lloyd's conclusion for the abscission cells of *Mirabilis*, that the free cells are not degenerating but have merely been set free by the dissolution of the cell walls. They are hypertrophied, but their internal structures, the nuclei, nucleoli, plastids, etc., appear quite normal though perhaps slightly enlarged. Such cells show streaming of protoplasm, and the starch in the plastids which is abundant at first gradually decreases and may entirely disappear after two or more weeks, indicating that cell metabolism is still going on. The cells ultimately die after the exhaustion of the reserve starch, but it is quite possible that they could continue to live indefinitely if kept under suitable environmental conditions. I have found free cells two or more weeks old which contained no visible starch; the plastids, however, had become green and were perhaps carrying on photosynthesis.

In a former paper by the writer (1927) it was noted that the plastids in the free cells are typically arranged in one or more bands in the primordial utricle. Plasmolytic experiments show that cross walls are not present there though they may appear to be on cursory examination. More careful study shows that the bands mark the contacts between vacuoles. As the vacuoles tend to round up, the cytoplasmic material aggregates around the periphery of the plane of contact between the vacuoles. The plastids under these conditions come to rest in this thicker zone of cytoplasm, but the larger size of the nucleus often prevents it from doing likewise. Although I have examined hundreds of these free cells both in the living condition and in prepared slides, I have never found them bi-nucleated or undergoing division.

The Protective Cork Layer

A protective cork layer is regularly formed along the inner margins of an intumescence and separates it from the normal tissue below. This may be

considered as a typical wound response for apple, since a layer almost identical in appearance often forms in the cut ends of twigs when merely exposed to conditions unfavorable to the production of callus. I have found them occasionally on cuttings which were kept in bell-jars in the greenhouse, and they can at times be observed on the cut ends of small branches of trees growing out-of-doors. In this case the drying of the injured ends has resulted in the death of the tip of the twig, so that the corky layer is bordered above by shriveled tissue rather than by the swollen tissue of an intumescence. When the zone forms in the cut ends of twigs in control bell-jars, it is bordered above either by apparently normal or by dead tissue. It is well known that protective layers similar to these develop in a great many species of plants in response to fungus invasions, toxic agents, and various wound stimuli.

The cork layer is formed, as are those described by Taylor (1919), Massey (1916), and others, by one or more divisions of the cells lying along the zone of the future protective layer. The oldest cells of the collenchyma may participate in its formation, so that the new cork finally connects with the dead cork of the stem (Pls. XXXIII and XXXIV, figs. 4, 7, 8, and 9). The layer may be from one to several cells thick and not infrequently forms a clump of meristematic tissue where it crosses bast fiber groups, as though sealing them off. This increase in thickness upon crossing the hard bast is shown by Sorauer (1909) for stems of sweet cherry which have formed a corky layer near the cut ends of branches. A period of ten to fourteen days is required for the cork itself to develop (fig. 7).

When conditions are such that the intumescences develop very rapidly, the plant may fail to form this protective layer (fig. 6). In this case the normal tissues and those which are undergoing disorganization are not sharply separated. When, on the other hand, conditions are less favorable to the formation of the intumescences (as with high temperatures), the plant forms the layer and stops further spread of the lesions. Under these latter conditions the zone of transition between the normal and disintegrated tissues is very sharp, often consisting of merely the protective layer.

One can outline the future limits of an intumescence even in its incipient stages of development. If marks are made with India ink around the base of an incipient swelling on the cut end of a twig it will be found two weeks later that the intumescence in its maximum development has not passed this line. It is evident therefore, that the position of the cork layer is determined within two or three days after exposure to the gas, and that its position remains unchanged during the rest of the period of development of the intumescence.

The corky layer most frequently extends more or less transversely across the bark from the phellogen to the true cambium. This is well shown at the base of intumescences in the cut ends of twigs (*A C*, fig. 9). It turns sharply in from the phellogen and outer cortex as a parabolic curve, which gradually

flattens out and approaches the true cambium asymptotically. This gradual approximation to a straight line on nearing the cambium is almost universally present in preparations of intumescences on the ends of cuttings. This characteristic curve may indicate something as to the diffusion rate of the ethylene or its stimulus as it penetrates the deeper regions of the phloem and cortex.

The protective layer cuts transversely through the bud-base in a plane some distance below the bud and connects with the phellogen surrounding it. The stimulated region is thus separated from the normal tissues below. As can be seen in figure 7, all the bud and most of the bud-base outside the cork may then be destroyed in the formation of the intumescence. The depth at which this corky layer develops in the tissue depends upon how favorable conditions are for the formation of the intumescences. When there are superficial mechanical wounds in the dead bark, as shown in figure 8, which facilitate the entrance of the ethylene, the plant may simply cut out the mass of tissue affected and no intumescence develops.

The evidence is clear that the corky layer actually prevents the unlimited spread of the disorganization of the tissues. I have never found any indication of hypertrophy in the tissues inside of the adventitious cork layer, even when disintegration external to it may be complete (fig. 7). It is interesting to note in this connection that cuttings which have formed these layers in response to exposure to ethylene may not respond again if placed in the presence of the gas, unless they are first wounded so the gas can penetrate.

DISCUSSION

The disorganization of tissues and the modification in the cells which accompanies the formation of intumescences in the buds and stems of apple are similar to those described by Lloyd (1916) as occurring during the formation of abscission layers in *Mirabilis*. In both cases highly differentiated tissues undergo changes which result ultimately in complete disorganization and the freeing of their protoplasts. The intumescences here studied are more extensive and may involve all the living bark, while the abscission layer studied by Lloyd is restricted to a narrow transverse plate of cells at the base of a petiole or in an internode. This difference between the two responses is, however, a difference in the total mass of tissue affected and not in the type of tissue or the fundamental processes of wall solution and hypertrophy and separation of cells.

I have emphasized (1926) that ethylene induces abscission almost universally in plants. In view of this characteristic and also in view of the similarity in the gross structural aspects of the disorganization processes, it is perhaps not surprising to find similarity in the changes involved in the development of intumescences and abscission layers. Parallel studies of the developmental stages of abscission layers induced by abnormal environmental conditions and those induced by traces of ethylene gas might be very instructive as to the basic similarity between the two responses.

Enzymatic activity is obviously an important factor in the formation of such intumescences. The localized corrosion figures of cell-wall layers described above, as well as those given by Lloyd for abscission cells of *Mirabilis*, are very suggestive of the figures by Jones (1909) showing digestion of the walls of carrot cells by the hydrolytic enzyme taka-diastase. I have suggested in previous papers that the effects of ethylene on plants may be, in many cases, a speeding up of the metabolic processes. The evidence of greatly increased enzymatic activity offered by the striking corrosion figures in these intumescences supports this view. I have, as yet, no exact quantitative and qualitative data on this problem.

The histological and cytological data on the fundamental changes in the tissue outgrowths described by Sorauer, Küster, Shilling, and others are insufficient to establish the exact relationship of these outgrowths to those here reported in the tissues of apple. From the data available, however, it would appear that the outgrowths in apple differ in several fundamental aspects.

A solution of walls, which usually occurs as a very irregular corrosion of the cellulose thickenings, plays a major rôle in the formation of the intumescences which I have studied. Shilling (1915), on the other hand, reports that a chemical alteration in the walls together with frequent divisions and very great radial enlargement of the cells are the chief changes which he induced in woody stems by treating them with paraffin. There is a bare possibility that the corrosion which is so characteristic for apple tissue represents a climax case of the wall alteration found by Shilling. The possibility is made more remote, however, by the solution of elements in the stems of apple, which remain unmodified in his material.

The buds, the bast fibers, and even the walls of the xylem vessels may be completely destroyed in the stems of apple by solution processes induced or accelerated by ethylene gas. I find no reference in the literature to a destruction of buds in plants in which intumescences have been reported. Likewise, the bast fiber groups in the intumescences studied by other investigators are reported to be unaltered, except for physical disarrangement. Both Sorauer and Küster describe a bowing out of the bast fibers by hypertrophy of the underlying tissues in the stems of *Ribes aureum* affected by dropsy disease. Shilling also gives numerous figures of extensive intumescences in the bark of woody stems, but without exception the hard bast elements are normal. He also states that these and other non-living elements such as latex tubes, resin ducts, and stone cells remain unchanged even though the parenchyma cells adjacent to or surrounding them may be greatly hypertrophied. A digestion of walls such as that which frequently, in the case of apple, results in the liberation of the spirals or other thickenings of the xylem vessels has not been reported by these investigators.

A cork layer separating the normal and diseased areas of the stems, and limiting the spread of the intumescences, seems to be entirely lacking in

the overgrowths described by Shilling. Further study may show that the zone of meristematic cells which he figured for *Syringa Emodi* as giving rise to overgrowth tissues in the phloem region was an incipient or poorly developed cork cambium. He very definitely states, however, that the proliferated layer is not a "cork mantle" because all the cells are living and rich in cytoplasm, and that in addition the meristematic layer itself contains small but distinct intercellular spaces.

The inhibition of callus formation reported in my first paper (1926) is probably but another expression of ethylene injury of plants. Of the twenty-eight species of woody cuttings tested which formed callus readily in the controls, only three exhibited the same degree of callus formation in the presence of the gas.

The development of the corky layer is apparently the cause of the inconclusive results obtained from experiments designed to test the penetrability of apple tissues and its effect on the intumescence response. I found (1927) that removal of the abscission layer at the base of the buds just before treating the cuttings with ethylene insured intumescences in almost every bud, but that scraping, notching, or slitting other parts of the stems did not necessarily cause like responses in those areas. This difference is attributable to the character of the tissues in the two regions. The tissues of the bud-bases contain very large intercellular spaces, which not only increase the possible rate of diffusion of the gas into the tissues, but also make it more difficult for the plant to form an unbroken cork layer. Consequently it is to be expected that the buds will usually form intumescences when the old abscission layer is removed. The swellings in the other regions of the stem develop several days later than those in the buds, thus allowing more time for the plant to establish a protective zone. Moreover, the smaller number of intercellular spaces facilitates the formation of a continuous corky layer. Mechanical wounding of the stems may, therefore, give rise to a corky layer which cuts out the mass of tissue which has been exposed to the gas as shown in figure 8, and this tissue may fail to form an intumescence because the cork has cut it off from its water supply.

SUMMARY

A detailed histological and cytological study shows that the intumescences which develop in the buds and stems of the "Transparent" apple in response to stimulation by ethylene gas arise through three fundamental changes in the tissues affected, namely, solution of walls, hypertrophy of cells, and proliferation of cells.

The walls are corroded away very irregularly by solution processes induced or accelerated by ethylene gas. Certain restricted portions of the secondary walls may be entirely corroded before adjacent areas are appreciably modified. The middle lamella goes into solution just prior to, or at the time of, the complete solution of the secondary thickenings. The solu-



tion of walls results ultimately in the more or less complete separation of the cells from tissue continuity, and in the rounding up of the individual protoplasts.

All living elements between the phellogen and the true cambium may undergo this corrosion of walls and the liberation of the cells from the tissue masses. Even the non-living elements such as the bast fibers and walls of young xylem vessels are often digested away.

Very distinct corrosion zones, which apparently represent diffusion tracts for the ethylene or the ethylene stimulus, are usually present in young intumescences.

Great hypertrophy of cells usually accompanies the solution of the walls, but this enlargement of cells is not necessarily the primary cause for the freeing of the cells from tissue continuity. The free cells in the intumescences may vary from normal ones only 25 by 30 microns to giant ones which are as much as 50 by 360 microns.

The phellogen frequently exhibits a more striking hypertrophy than any other tissue of the stem.

The cells of any living tissue of the bark may divide during the formation of the intumescences. Only normal mitotic figures were observed. An increase of about 33 percent in the number of cells was found to occur in the intumescences in the ends of cuttings.

Calcium oxalate crystals are usually very abundant among the free cells of an intumescence, and are apparently a by-product of the solution of the cell walls.

The thin-walled hypertrophied cells, which make up the major portion of the swollen mass of the intumescences, usually contain two or more large vacuoles. The cytoplasm between these vacuoles makes the cells appear to be divided by cross-walls. These cells may live for several weeks after becoming free from the tissues.

A protective cork layer generally forms along the inner margin of an intumescence and separates it from the normal tissue below. This layer when present limits the spread of the intumescences. When conditions are very favorable for the development of the swellings the cork layer may fail to form.

The present study was conducted while the author was a National Research Council Fellow in Botany. He wishes to express appreciation to Professor R. A. Harper for the interest he has taken in this study and for many helpful suggestions.

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DESCRIPTION OF PLATES

The drawings and photomicrographs were made from "Transparent" apple (*Pyrus Malus*) except figure 6, which was from cultivated crabapple. The drawings were made with the camera lucida, and a Zeiss apochromatic lens combination.

PLATE XXXIII

FIG. 1. A single cell from collenchyma, showing the appearance of walls undergoing corrosion. Middle lamella is distinct in unmodified parts of wall. Lightly stained walls at base are in advanced stage of modification. *b*, lightly stained, modified wall; *d*, one of three intercellular spaces left on solution of walls. $\times 1000$.

FIG. 2. Photograph of cell shown in figure 1. Middle lamella is very distinct in unmodified part of wall. Note that the protoplasts do not protrude into the intercellular space (*d*). $\times 1250$.

FIG. 3. Radial section showing phellogen undergoing hypertrophy. The transverse walls of the enlarging cells gradually become thinner as the modification of the wall progresses. There is little or no evidence of the corrosion of the walls which is apparent in figure 4. $\times 400$.

FIG. 4. Radial section through collenchyma directly beneath the phellogen, showing general character of the intumescence in stems of "Transparent" apple. *AC*, adventitious cork cambium separating the normal and swollen tissues; *P*, phellogen; *a*, heavily stained, unmodified wall; *b*, lightly stained, modified wall; *c*, unstained wall just prior to complete solution; *d*, intercellular space left on complete solution of the wall. The dotted lines indicate possible diffusion tracts for the ethylene or the stimulus it initiates. $\times 400$.

PLATE XXXIV

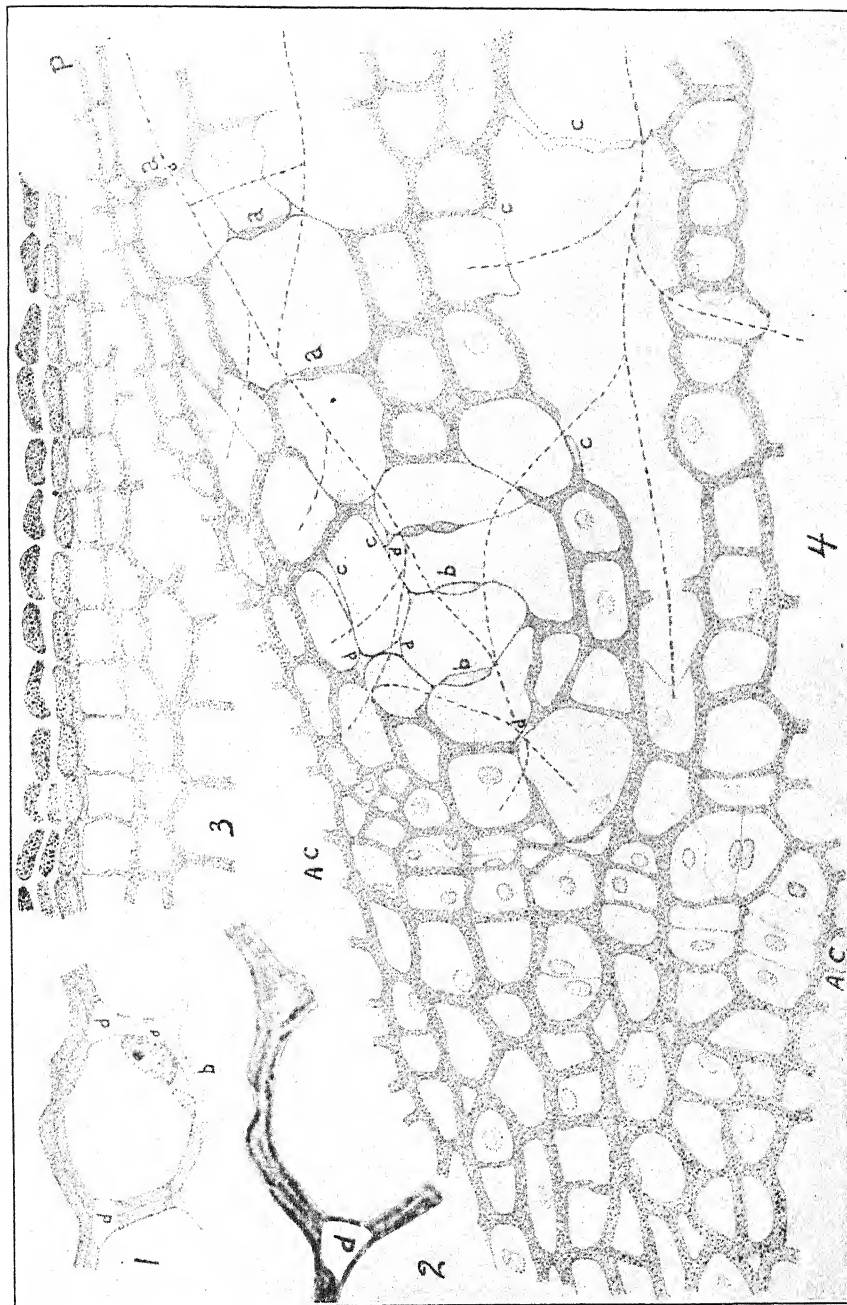
FIG. 5. Photomicrograph of collenchyma and cork, showing the hypertrophied phellogen. Note that the collenchyma as yet is unaffected by the gas. $\times 120$.

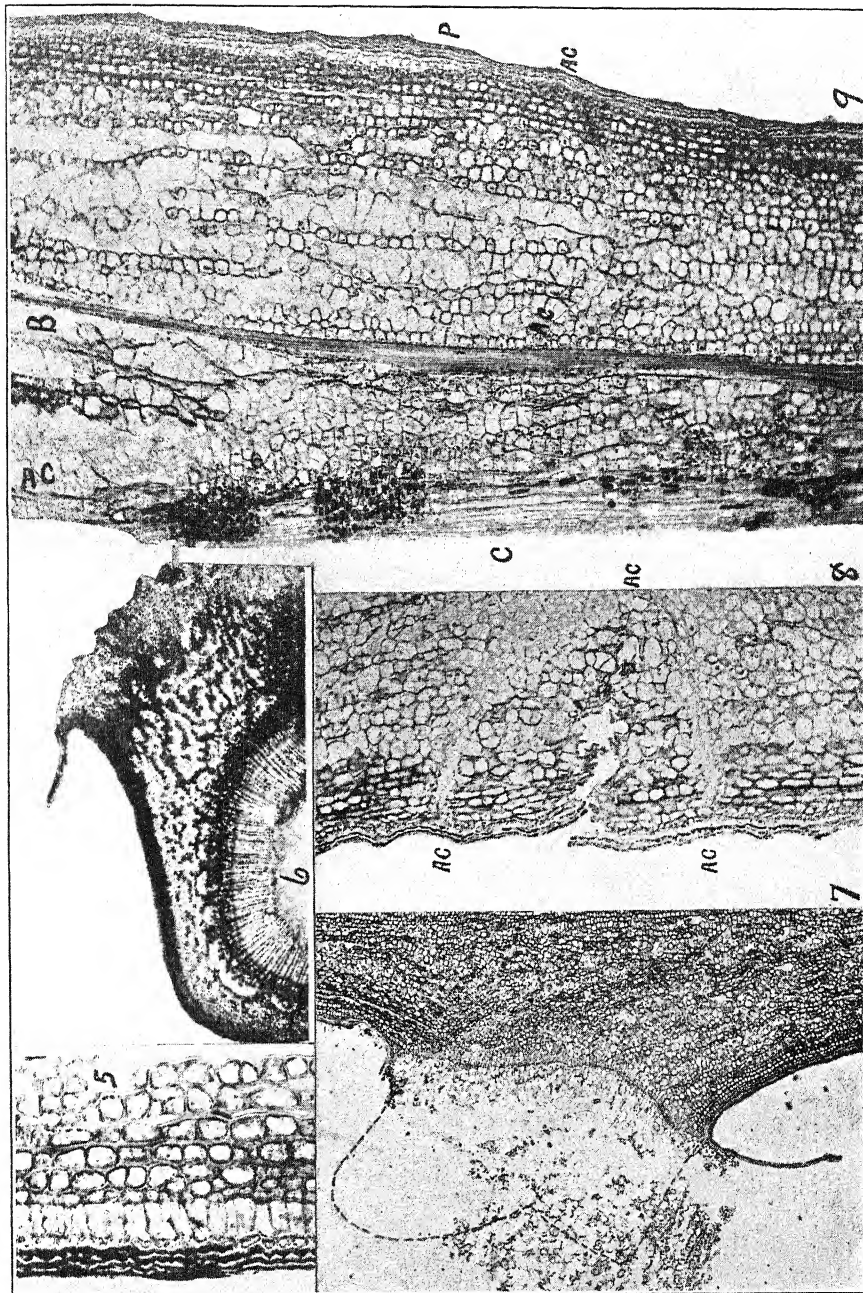
FIG. 6. Photomicrograph of a transverse section through a stem of cultivated crabapple, showing general character of the intumescences. The crescent-shaped groups of bast fibers to the left of the section are normal while those to the right are entirely gone. No cork layer is present. $\times 20$.

FIG. 7. Photomicrograph of a bud-base which has been sealed off by a cork cambium. A layer of cork two to three cells thick has become differentiated. The dotted line indicates the approximate extent of bud and bud-base before the intumescence developed. $\times 22$.

FIG. 8. Photomicrograph of a radial section through collenchyma and dead cork, showing a superficial mechanical wound around which cork is forming. The cambial layer (*AC*) is present, but the cork has not formed. $\times 64$.

FIG. 9. Photomicrograph of radial section through normal and swollen region of the bark of "Transparent" apple, showing the general characteristics of the intumescences. The adventitious cork cambium (*AC*) is forming a cork layer separating the normal and diseased tissues. *P*, phellogen (note that it has hypertrophied in some places); *C*, true cambium; *B*, bast fiber group. $\times 565$.





THE MOLECULAR STRUCTURE OF THE CELL WALL OF FIBERS. A SUMMARY OF X-RAY INVESTIGATIONS

O. L. SPONSLER

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During the past decade a method of studying atomic and molecular structures, physically, has been developed which is based upon the diffraction of X-rays from layers of atoms. It has been applied to many substances, especially to crystalline materials, and through its use enormous strides have been made in gaining a clearer insight into their structure. The same method has now been used to study the structure of the less obviously crystalline material of the cell wall in order to determine, if possible, the kind and arrangement of its molecular components. An account of this work is presented in the present paper.

THE STRUCTURAL UNIT

If it were possible to magnify a fragment of a cell wall many thousand times, enough so that the constituent "building bricks" or structural units would become visible, each unit would appear as an irregular group of atoms. In a sense the X-ray method makes it possible to do this and to make the structural units visible, but visible to the mind rather than visible to the eye. In the following account an attempt is made to produce a picture of such a group of atoms; to show how groups of this kind are built into long "string-of-beads" structures; and to describe their arrangement in the cell wall. With this descriptive account will be given an explanation of the methods by which it has been possible to determine a sufficient number of the characteristics of the groups to enable one to visualize them. The explanation will not go into great detail, the effort being to develop the picture rather than to demonstrate the proof of it. For the latter, references to more detailed papers are given. The soundness of the conclusions will also be considered briefly.

The portrayal of this structure is made possible chiefly through work done with X-rays, using ramie fibers as the source of cell wall material. The methods employed are commonly spoken of as crystal structure methods. Although they are not difficult to understand, explanations of them are likely to become considerably involved, and an attempt will therefore be made here to keep these explanations as simple as possible.

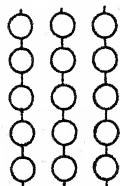
The irregular group of atoms which was mentioned as the building brick of the cell wall has been shown to resemble a glucose molecule in that the former is a $C_6H_{10}O_5$ group and the latter $C_6H_{12}O_6$. The location of the

atoms within these groups is very much the same in both cases. While it is necessary to keep in mind the conception of the molecule as a group of about twenty atoms, it is extremely convenient to think of it as a unit in itself, with a definite although irregular shape, having length, breadth, and thickness, or at least having space allotted to it in three dimensions. We need not be concerned about the structure of the constituent atoms, but may consider them merely as particles which are called carbon atoms, oxygen atoms, and hydrogen atoms and which are recognized as occupying space and having weight. This group, $C_6H_{10}O_5$, having definite dimensions and weight, may be thought of as a structural unit which keeps its shape, size, and weight wherever it is found in the cell wall, just as an ordinary brick remains the same regardless of where it is placed or how it is oriented in a brick wall.

ARRANGEMENT OF UNITS IN THE WALL

If magnified many thousand times, this group would appear to be attached to similar groups to form a long chain. The chain would be straight and would be one of many similar chains all lying parallel to one another lengthwise in the wall. In fact, these chains would form the wall, for there would be nothing between them. In order to help visualize this structure, text figure 1 is used to represent a piece of the cell wall consisting of three short lengths of chains. Each length is made up of only five of an indefinite number of $C_6H_{10}O_5$ groups which are represented here by circles.

It has been found that these chains extend lengthwise of the fiber and are more or less parallel with its long axis, as well as being parallel to one another. Further, the distance between these parallel chains is very uniform, although very small. It must be realized that such distances are far below microscopic visibility and that a chain having 2000 of these



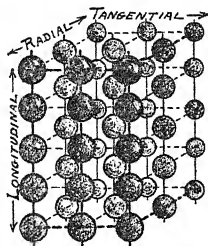
TEXT FIG. 1.

structural units in its length would be only one micron long. The smallest particle visible in the microscope would contain a very great number of these $C_6H_{10}O_5$ groups.

The chains in text figure 1 may be thought of as extending the full length of the fiber, all parallel with and separated from one another by uniform distances. One layer of these chains may be considered as forming the outside layer of the fiber wall, thus becoming more or less cylindrical; another layer or cylinder as occurring just inside this, a third cylinder inside the

second, and so on, cylinder inside of cylinder concentrically making the wall several thousand cylinders thick. The thickness of any one of these concentric cylinders is the thickness of one $C_6H_{10}O_5$ group; the diameter, about that of the fiber.

A very minute piece of this fiber wall may be represented in perspective by text figure 2, where each circle represents again a whole $C_6H_{10}O_5$ group. The dotted lines are used merely to outline the cubical block which is composed of three layers of chains, as though the block were a piece from the

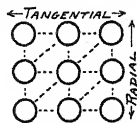


TEXT FIG. 2. Diagram of a minute block taken from the cell wall, shown in perspective. Each circle represents a whole $C_6H_{10}O_5$ group. All groups are identical regardless of the size of the circle. Pieces of nine chains are shown forming three tangential layers of three chains each, so arranged that they also form three radial layers of three chains each. There are five unit groups in each chain.

wall which happened to go no deeper than through three of the concentric cylinders. It happened also to contain only three chains of each cylinder and only five structural units of each chain.

This figure is intended to bring out two points; first, that the chains are arranged to form radial layers, as well as tangential layers, of three chains each; and second, that if we think of both these sets of layers as occurring vertically in our perspective picture, then there are horizontal layers also, five of them in this case. These are layers of units rather than of chains, but of course the radial and tangential layers are of units also, although attached in chains.

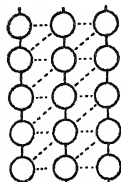
This structure, with layers in its three different dimensions, is called a *space lattice*, or more strictly a three-dimensional space lattice. An inference



TEXT FIG. 3. A view of text figure 2 looking lengthwise of the chains. Each circle represents the end of a chain.

which may be obtained from it is that each unit is very accurately placed in the cell wall and perhaps is oriented in a particular way with respect to its neighboring units.

When only the end view of text figure 2 is considered, a view such as would be obtained from the cross section of a fiber, the ends of the chains would occur somewhat as in text figure 3, where each circle represents the end unit of each chain, the other units being hidden behind it. Here in addition to the three tangential and three radial rows, various diagonal rows also become evident, as indicated by the dotted lines.



TEXT FIG. 4. A tangential or radial view of text figure 2.

In text figure 4 a side view of text figure 2 is shown. It corresponds to a longitudinal view of a fiber and may represent both of two commonly considered views, the radial and the tangential. The three vertical rows are rows of chains running lengthwise of the fiber; the five horizontal rows are rows of units not linked together into chains. Dotted lines indicate diagonal rows here also.

A very brief summary may be of value at this point. The cell wall of fibers may be thought of as built of long chain-like structures in which the constituent units, $C_6H_{10}O_5$ groups, correspond to the links of the chain. The chains themselves are laid parallel to one another and extend lengthwise of the fiber, forming layer after layer, quite uniformly spaced, from the outer to the inner surface of the cell wall.

EXPERIMENTAL METHODS AND PROCEDURE

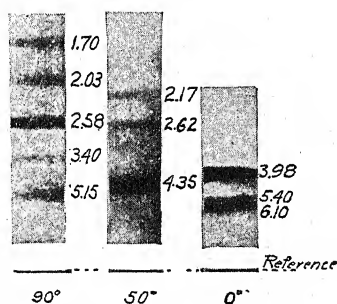
The procedure used in demonstrating this structure includes several experimental and interpretative methods which are not commonly employed in botanical investigations. They will be explained later, in part. In brief, the steps in the procedure were as follows:

1. Plant fibers were subjected to X-rays in an appropriate apparatus, (6, 4, 8) and were found to produce lines on a photographic film.
2. These lines, which form what is called a *diffraction pattern*, were interpreted as representing a space lattice (9, 11), somewhat as shown in text figure 2.
3. The unit of that lattice was then studied in connection with the $C_6H_{10}O_5$ group.
4. Models of C_6 groups were constructed to scale and built into the form of a lattice. They were then studied in more detail in an attempt to reconcile the chemists' data with the X-ray data (11).
5. Studies of phenomena of swelling (12) and of certain physical properties of fibers were made in connection with the structure that had been developed.

Although several different kinds of fibers and cells were used, the work was carried on in greatest detail with bast fibers of ramie (*Boehmeria nivea*); in much less detail with hemp, flax, and spruce fibers and cotton hairs; and only in an exploratory way with the more iso-diametric cells such as those of pith. The conclusions arrived at, then, must apply specifically to the ramie fibers. Other fibers seem to have the same structure, but as yet that has not been demonstrated with certainty. Practically nothing can be said of the wall structure of any other cells than those of the elongated fiber type.

The method of investigation demanded the use of a small compact bundle of many fibers (8). The long, straight, smooth fibers of ramie were adapted admirably to building up such a bundle in which most of the fibers were laid parallel, or nearly so. That was a decided advantage, for it then became possible to use the bundle as though it were a single fiber which could be turned in various positions for study. Tangled or matted fibers present almost insurmountable obstacles, or at best are capable of much less certain results.

As to the X-ray apparatus used and its manipulation only a few words need be said for the details are fully described in other papers (7, 11). It has been shown by a very great amount of work with crystals, on the part of many investigators (13, 1), that whenever atoms or molecules are arranged in layers,

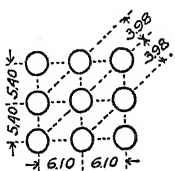


TEXT FIG. 5. Reproduction of X-ray diffraction patterns taken with the bundle of ramie fibers at three different angles to the X-ray beam. The one marked 0° was taken with the fibers parallel to the direction of the beam; that marked 90°, with the fibers at right angles to the beam; that marked 50°, with the fibers at an angle of 50° to the beam. Figures opposite each line give the distance in Å.u. between the atomic layers of the set which produced that line.

parallel to and uniformly spaced from one another, they are capable of "reflecting" X-rays to produce a diffraction pattern. The principles involved in this do not lend themselves readily to a simple description. The lines which make up the pattern or *spectrum*, as it is sometimes called, are shown in text figure 5 where photographs of the fibers from three positions are reproduced. The pattern marked 90° was taken when the fibers were

turned so that the layers of atoms extending across the fibers, at right angles to the long direction, were in position to produce diffraction lines. The one marked 0° has its lines associated with longitudinal layers, and that marked 50° , with certain diagonal layers.

There are several points which should be understood, to gain a clear conception of the significance of X-ray diffraction. Certain requirements must be met in order that a substance shall be capable of producing a diffraction effect. A layer of atoms or molecules, such as exists on the face of a crystal, reflects X-rays somewhat as it reflects visible light, except that only a very small amount of the X-ray beam is reflected from the surface layer. The greater part of the beam passes on into the crystal where each layer parallel to the surface reflects an equally small amount. When there are a suf-



TEXT FIG. 6. Same as text figure 3 with the distances between the layers of chains given in Ångstrom units.

ficiently large number of layers to make the sum of all their reflections an appreciable amount, a diffraction line is possible. Not only must the *number* of layers be large, in the thousands usually, but they must be *parallel* and the *distance* between every two must be the same. When those three conditions are fulfilled the layers are capable of producing *one* line of a diffraction pattern. Another face of the crystal may have as *many parallel* layers beneath it, but the *distance* between the layers may be different from that of the crystal face just considered; these layers then are also capable of producing *one* diffraction line, but that line would have a different position in the pattern. In other words every line of the diffraction pattern corresponds to its *own* set of parallel layers which have a very *definite distance* between them. This may be illustrated by text figure 6, which is the same as text figure 3 except that dimensions in Ångström units are given (1 Å.u. = 0.000,000,1 mm.). Three sets of layers are indicated in the figure, spaced respectively 6.10, 5.40, and 3.98 Å.u. The diffraction pattern would have three lines, one for each set, as shown by the lines in the 0° photograph of text figure 5.

RESULTS AND INTERPRETATIONS

Diffraction patterns obtained from the ramie fibers had over thirty definitely measurable lines (8). That proved directly that the *atoms or molecules are arranged in parallel layers, in many sets of layers which have different spacings*. It was readily demonstrated, by turning the bundle of fibers to different known positions, (a) *that certain sets of layers extended*

lengthwise of the fibers, (b) that other sets of layers extended across the fiber wall at right angles to the lengthwise layers, and (c) that still other sets formed definitely determined angles with the lengthwise layers. The only conclusion allowable in the present state of knowledge is that structural units of some kind form a space lattice in the wall of the fiber.

From the photographic record of these lines it is possible to compute the distance between the layers of each set (8). In text figure 5 the figures placed opposite a given line indicate the computed distance, in Ångström units, between the atomic layers of the set which produced that line. The pattern marked 90° shows definitely that several sets of layers extend across the fiber; that marked 0° shows the existence of at least three sets which extend lengthwise of the fiber at right angles to those from the 90° pattern; and the 50° pattern shows several more sets which make a 50° angle with the long axis and therefore with the layers which produced the lines of the 0° pattern. By fitting these layers together a three-dimensional structure somewhat like that represented by text figure 2 was finally determined in which every one of the thirty odd sets of layers was found to fit (9). That means that each set had its place in the lattice where the distance between layers, as determined from the photograph, was the same as the distance which was computed from the dimensions of the lattice. The observed and computed figures had to agree within 1%, rarely as high as 2%, in order to be considered satisfactory. Further, the angle which a layer made with the long axis of the fiber, as determined from the apparatus, had to be the same as that which could be computed from the lattice. Here it was not possible to obtain as consistent nor as great accuracy, because the position of the fibers which were active in reflection could not be determined within several degrees. However, in most cases satisfactory agreement was obtained.

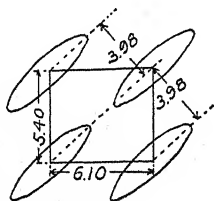
It seems highly probable that the lattice thus constructed is the correct one since all of the observed data, consisting of over thirty diffraction lines, are in agreement with the geometrical characteristics of the lattice. Even if no other evidence were brought forward this lattice would still be considered as being well established. But other evidence is forthcoming, perhaps slightly less direct in its nature but nevertheless worth consideration. The lattice, as such, has comparatively little meaning in itself. It is the information which can be deduced from it that is of importance. Once the lattice is determined it then becomes possible to study the unit, or building-brick, of which it is constructed and to introduce evidence of a somewhat different nature to help prove the correctness of the structure.

Each unit of the lattice, as indicated by a circle in text figure 2, has allotted to it a certain amount of space which is called here, for the time being, the *elementary cell*. The volume of this space is $5.15 \times 5.40 \times 6.10 = 169$ cu. Å.u., as computed from the distances (9) between the layers. This, then, is the volume of the group of atoms which acts as the unit of the lattice. According to the cellulose chemist that group is the anhydro-glucose residue,

$C_6H_{10}O_5$. It should have a volume equal to that of the elementary cell and it should have dimensions which would allow it to fit into that cell. It is possible to test out both of these requirements.

The volume of the $C_6H_{10}O_5$ group as it occurs in a cellulose fiber bears a definite relation (*a*) to its molecular weight, (*b*) to its specific gravity, and (*c*) to the Avogadro number. All three of these are known or are readily obtainable, and from them the volume of the unit group has been determined (9). It is 170 cu. Å.u. This compares so favorably with the volume of the unit of the lattice, 169 cu. Å.u., that it seems highly probable that the chemists' C_6 group and the structural unit determined by X-ray methods are identical.

In order to determine the dimensions of the $C_6H_{10}O_5$ group, the chemists' data were very carefully studied (11) and the position of the atoms within the group with respect to one another, which seemed to fit the evidence best, was selected as a pattern from which to construct a model of the group. Atomic diameters were obtained from the works of various investigators (13, 1) on crystal structure, and a large model was made to scale. Even



TEXT FIG. 7. A view similar to text figure 6 except that only four of the chains are represented, each by an elliptical figure which more nearly represents the general outline of an edge view of a $C_6H_{10}O_5$ model.

though this model had no dimensions whatever taken from the X-ray fiber study, it was an excellent fit in the elementary cell of the lattice. What was still more striking was that it would fit in only one position and that position was the one which seemed to be demanded by the X-ray data (11). At almost every turn new points of agreement were brought out, only a few of which will be mentioned here.

The model was a flattish structure, wider than it was high. Its outline was that of an irregular disc. When attempting to fit it into the elementary cell it was found that there was not room enough on the sides of the cell, but when placed edgewise on a diagonal as shown in the diagram, text figure 7, its dimensions agreed satisfactorily. It was interesting to note that when in this position it accounted (11) for the intensity of the strong and the weak diffraction lines which are shown in the 0° spectrum of text figure 5.

The orientation of the unit given in text figure 7 accounts for its thickness dimension and its width, and forces its third dimension, which we have called its height, to come within 5.15 Å.u. along the long axis of the fiber. Surprisingly, the height of the model corresponds almost exactly to that distance.

This very close agreement suggests a different inquiry. If the units fit so closely together lengthwise of the fiber as indicated by the height dimension, may it not be possible that they are directly attached to one another? The other two dimensions allow for appreciable space between units in the lattice, but here, lengthwise of the fiber, there is no extra space. When the diffraction patterns are examined they are found to indicate a direct connection or attachment between the units in the longitudinal direction (8). From the chemists' data (5) we find that this C_6 unit or cellulose group is not an independent unit, if we may use such an expression here, but is rather colonial in its habits. The chemist calls it an anhydro-glucose residue, meaning that it is the residue or part left when a molecule of water is taken from a molecule of glucose. As a residue the bonds which had attached the H and OH of the water molecule are left dangling or unsatisfied when the H_2O is removed. Its tendency then is to unite to something else through those unsatisfied bonds. In our lattice of text figure 2 the only thing that one of the unit groups can attach itself to is another C_6 group, and if it should happen that the two loose bonds are at opposite sides of the group it could attach itself to two other units, to form a chain of three, with a loose bond at each end. That would permit the formation of chains of indefinite length. From the lattice it would seem that these groups could be attached to one another laterally as well as vertically, but the X-ray data indicate very strongly that if there is any attachment it must be into vertical chains (11). The chemists are fairly well agreed that the units are linked to one another in some way, and that they are linked through an oxygen atom to certain definite carbon atoms (2) which have been numbered 1 and 4. It happens that these two carbon atoms are exactly opposite each other, which makes it possible for the units to become linked together into straight chains and to form a structure such as we have indicated in text figure 2.

RELATION OF STRUCTURE TO CHARACTERISTICS OF FIBER

Although all of the conditions are satisfied,¹ additional confirmation is obtained from the fact that when several models of the unit group are fitted together into the dimensions of the lattice, the properties of the fiber agree in

¹ After this paper had gone to press there appeared a paper by K. H. Meyer and H. Mark, on the structure of the crystalline constituents of cellulose in *Ber. Deutsch. Chem. Ges.*, April, 1928, 593-614. From somewhat different methods these authors arrive at the same conclusions reported in our earlier papers (8, 9, 11) in so far as the fundamental points of our structure are concerned; namely that glucose residues with amylenic oxid ring structure are attached to one another through oxygen bridges to form long chain molecules; that the chains, parallel to one another, extend lengthwise of the fiber; and that the glucose residues act as units of a lattice structure. There are a few minor points on which agreement has not yet been reached, such as the spacing of the chains laterally and the orientation of the glucose residue in the chain; but on the whole, we may now feel that the conception of a lattice with the chain structure is well established for the cell-wall structure of fibers.

many ways with predictions which may be made from the structure. A few of these will be discussed here.

There seems to be a fairly clear correlation between the forces existing in three directions in the lattice and the strength values, swelling properties, and thermal expansion values in three directions in the fiber. The primary valence bonds which hold the groups into a chain would make for greater strength lengthwise of the fiber than would the secondary valences which hold the chains to one another laterally. The fiber could swell laterally much more than longitudinally if it could swell at all in the longitudinal direction (12). Molecular agitation, which is associated directly with the coefficient of thermal expansion, would be greater laterally than lengthwise of the fiber (11). Data concerning these three properties are in good agreement with the lattice structure, at least qualitatively. Up to the present time no attempt has been made to show a quantitative relation.

The chain structure seems to account also for certain observations in the chemistry of cellulose (11) and in the mercerization of cellulose fibers (12). It explains why certain addition products of cellulose may be formed without altering the fiber appearance; and why, when other esters are formed, the fiber disintegrates or goes into solution. In the process of mercerization, X-ray spectra show that the chains have been moved bodily to slightly different positions. The movement seems to be a lateral shift of the chains in such a way that after the treatment the chains are closer together in one direction and farther apart at nearly right angles to that direction. The units, however, have not been shifted from their position in the chain.

A few questions come up to which the lattice structure gives a negative answer. For example, there seems to be now no necessity for assuming the existence of micellae as *structural units of the cell wall*, that is, large units made up of many molecules, such as Nägeli proposed some sixty-five years ago; but the subject involves too many details to be taken up here.

In one of the recent reports of X-ray investigations two substances were assumed by Herzog (3) to be present in cellulose walls in order to explain certain diffraction interferences which would not fit into his lattice. That this interpretation was not valid has recently been shown by the author (10).

The extent to which this chain structure exerts an influence on the fiber markings so commonly seen in the microscope is probably not very great. This is evident especially when one recalls that a thin cell wall only one micron in thickness would require between 2000 and 3000 chains laid side by side to make up the one micron. Markings such as spiral thickenings or thickenings of any kind that are large enough to be visible would involve an enormous number of $C_6H_{10}O_5$ groups, the number reaching into the billions for a piece as small as a cubic micron. This makes it seem extremely probable that the inherent properties of the C_6 group would have little to do with the shape of structures which could be built from those units. It seems much more likely that the forms of visible markings are due to local

protoplasmic activities rather than to individual peculiarities of the $C_6H_{10}O_5$ group of atoms. A satisfactory discussion of this problem would be too long for the present paper.

Another suggestion which comes out of this concept of chain structure is concerned with the way in which the chain can be built up lengthwise. If a condensation takes place through which the glucose molecule is transformed into a unit of cellulose, an anhydrous residue, that condensation can take place only at a particular point for each molecule and only when the glucose molecule is oriented into a certain definite position with respect to the neighboring C_6 groups of the wall, and still further only through the action of a third body. The assumption is made that a glucose molecule is transformed into an anhydrous residue which at the same time becomes a part of a long string-like molecule of cellulose. It would seem that these long molecules of cellulose are not formed first and then placed in the wall but are formed in place as a part of the process of building the wall. The third body referred to must be very intimately associated with the protoplasm at the interface where the wall is being formed. It seems probable that the third body is the protoplasm itself and that it is in a condition, peculiar to itself at the interface at that moment, to carry out the condensation reaction the result of which is the transformation of a glucose molecule into a structural unit of a cellulose molecule and at the same time into a unit of the wall of the fiber.

SUMMARY

1. X-ray diffraction patterns prove the existence of structural units in the cell wall of ramie fibers.
2. They prove that these units have a regular arrangement in layers in the wall; that the layers are uniformly spaced; and that some of the layers extend lengthwise of the fiber and some crosswise at various angles.
3. From the patterns, the distances between the layers of the various sets of layers were computed.
4. The data obtained made it possible to construct a space lattice from which the dimensions of the structural unit were determined.
5. The unit was shown to be, with a very high degree of probability, a $C_6H_{10}O_5$ anhydro-glucose residue.
6. Evidence from the X-ray patterns, from cellulose chemistry, and from the models of the structural unit, makes it seem very probable that the units are attached in chains of indefinite length.
7. A summation of all the evidence indicates clearly that the structural units are $C_6H_{10}O_5$ groups attached into chains which extend lengthwise of the fiber, parallel to one another, and very uniformly spaced.
8. This structure is in agreement with all of the properties and reactions of cellulose fibers so far considered.

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COMPOSITION OF FUNGUS HYPHAE I. THE FUSARIA¹

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INTRODUCTION

The composition of the cell walls of fungal hyphae is imperfectly understood. In this respect there is a marked contrast between our knowledge of fungi and of the higher plants.

In view of the fact that some of our most serious diseases, such as the wilts caused by fusaria, are not amenable to methods of control in current use, it is reasonable to believe that a knowledge of the composition of the mycelia of these parasites will aid us in devising specific control measures. Furthermore, it sometimes happens that the identification of a fungus is very uncertain in the absence of fruiting bodies. In such cases a determination of chemical structure may be of value in detecting genetic relationship.

No reports of investigations on the chemical structure of the hyphae of any of the representatives of *Fusarium* have been found. This study is based exclusively upon that group.

HISTORICAL REVIEW

It would appear that more attention was given to the chemical study of fungi forty years ago than at the present time. De Bary (2) used the term fungus-cellulose, for a substance in many fungi which gave a different response to tests from any of the known substances found in the cell walls of higher plants. While he recognized that a cellulose-like substance was present in many species, he considered it to be in a modified form and not true cellulose. On the other hand, de Bary states that Richter saw no need for such a special designation because he considered the cellulose present in fungi to be infiltrated or mixed with other substances, possibly of an albuminoid nature. By the use of appropriate solvents such material could be removed and the cellulose demonstrated. He showed that certain species of *Claviceps*, the Polyporaceae, *Daedalea*, and the Agaricaceae, after prolonged maceration in a 7- to 8-percent aqueous solution of potash, readily gave a blue color with iodine and sulfuric acid, which indicated the presence of cellulose. Likewise, after maceration for 2 or 3 weeks the cellulose could be removed by dissolving it out with an ammoniacal solution of cupric hydrate in which this substance is readily soluble.

In spite of the observations of Richter, the term fungus-cellulose has been retained in literature since the time of de Bary, although its meaning has

¹ Published with the approval of the Director of the Ohio Agricultural Experiment Station.

never been clearly defined. At the same time we have been at a loss to account for the marked resistance which the mycelia of many fungi manifest against the action of strong acids, and for the fact that they are inert toward dyes and do not readily give tests for any of the cell-wall substances, such as cellulose, lignin, suberin, cutin, and pectic compounds, associated with the higher plants.

This is not the case, however, with all fungi. De Bary was the first to point out the presence of cellulose in the membrane of the Peronosporaceae, Saprolegniaceae, *Protomyces macrosporus*, and the young mycelium of certain mucors. It is interesting to note his observation that in such forms as *Clavaria juncea*, *Anihina pallida*, *A. purpurea*, and *A. flammea*, the presence of cellulose is sometimes indicated by iodine and sulfuric acid, but not always.

Mangin (6, 7), while agreeing with the observations of de Bary, states that the presence of normal cellulose is rare in fungi. He adds, however, that the structure of the fungus membrane is much more complex than has generally been suspected. In the Peronosporaceae he found cellulose associated with callose. He also pointed out that the cellulose of the parasite is much more resistant to agents of putrefaction than that of the host, and is insoluble in Schweitzer's reagent. Previous workers, according to Mangin, explained these facts by considering the cellulose of *Peronospora* to be in a different state of aggregation from that of the higher plants, thus accounting for the high degree of resistance which it possessed. Tanret (16) states that Fremy characterized it as metacellulose, Tschirch as mycine, de Bary as fungus-cellulose, and Braconnot as fongine. Mangin was able, by long treatment with Schweitzer's reagent, to remove completely the cellulose and to demonstrate the presence of a new substance to which he gave the name callose.

In the mucors, Mangin (8, 9, 10, 11, 12) reported the presence of cellulose, callose, and cutin. His characterization of the last substance is somewhat indefinite and one is left in doubt as to what he really had. Reference is made to the heavy incrustation of calcium oxalate crystals which appear to be associated with the mycelial filaments. The cellulose in the young hyphae and in the young sporangia is associated with pectic compounds. At maturity, or a little before, a change takes place. In *Mucor Mucedo*, at this time, neither cellulose nor pectic compounds can be detected. The peripheral region is nearly pure callose. These same observations were found to be true for *Phycomyces nitens*, *Rhizopus nigricans*, *Thamnidium elegans*, and *Mortierella polycephala*. Callose was also found in different species of *Chaetocladium*. In *Pilobolus* it was detected only in the lower portion of the sporangia, while the upper part was cutinized.

Callose has been found in the spores of *Mucor Mucedo*, *Rhizopus nigricans*, and *Phycomyces nitens*. In many other species the spore membrane was found to be inert toward dyes, yet Mangin was inclined to consider it as

resembling callose, although he was unable to determine the true nature of the substance to his full satisfaction. Chlamydo-spores, arising from the vegetative mycelium, were found to be inert toward the dyes which are fixed by cellulose and the pectic compounds, but, after maceration in hydrochloric acid and potassium chlorate, callose was found to be present. Callose was found at the base of the conidia and was thought to play a part in their dissemination.

In the zygospores two membranes were distinguished, enclosing the copulating cells cut off to form the gametes. The membrane of the copulating cells is very thin and has the same structure as the spore-bearing filaments, being composed of cellulose and pectic compounds. The episore is made up of cellulose impregnated or covered with albuminoid material, but after treatment with oxidizing reagents the reactions of cellulose are easily obtained. The type of cellulose was thought to be of a more resistant kind than that of the filaments, and in both cases to be distinctly different from the cellulose found in the phanerogams and vascular cryptogams. No callose was observed in the zygospores.

Judging from the variety and character of his reports, Mangin pursued the most extensive and detailed investigation of the nature of the mycelia of fungi. He relied to a considerable extent upon the selective property of fixing certain stains which various cell-wall constituents were assumed to possess. This undoubtedly led him into error in some of his conclusions. Few dyes are sufficiently selective in their reaction to justify one in placing very much dependence on them, yet they serve as valuable guides in the investigation of cell walls.

We clearly gain the idea, however, from Mangin's work that the walls of fungal cells are complex in structure, and that in this fact lies the secret of their remarkable resistance to reagents, and particularly to the action of strong acids. He also maintained that the presence of nitrogenous matter or of pectic compounds frequently prevented the penetration of reagents and accounted for the failure to secure tests for substances actually present. In this respect he was in agreement with Richter, who advanced the same idea many years before. With this conviction in mind he was the first to propose that such terms as meta-cellulose or fungus-cellulose be discarded.

It is difficult to understand why Mangin reports cutin or a cutin-like material in the hyphae of the Mucorales. He obtained no positive tests for such a substance. The wall was inert toward such stains as cyanin and alkannin, and readily dissolved in boiling nitric acid; whereas the cutin of the higher plants exhibits marked resistance to the action of strong acids and is readily stained. Although recognizing these discrepancies, he nevertheless considered the cell-wall substance which he found in the mucors as a transition form of cutin. It is probable that his so-called cutin-like material was chitin which he did not recognize, although Gilson (3) and Winterstein (18) had discovered and reported this in fungi about five years earlier.

MATERIALS AND METHODS

In this investigation the following twelve representative strains of *Fusarium* were used: *F. Lycopersici*, *F. oxysporum* var. *Solani*, *F. hyperoxysporum*, *F. cubense*, *F. sp.* isolated from raspberry, *F. sp.* isolated from snap bean, *F. sp.* isolated from asparagus, *F. roseum* isolated from wheat, *F. moniliforme*, *F. conglutinans*, *F. sp.* isolated from aster, *F. sp.* isolated from onion.

When grown upon potato dextrose or nutrient agar media, the differences of growth were sufficiently marked to consider them distinct strains. Particular attention was given to those isolations whose pathogenicity had not been tested.

The cultures were grown upon Richards' solution. Flasks of 300 cc. to 500 cc. capacity, containing respectively 75 to 150 cc. of culture medium, were used. The cultures were allowed to develop for two to three weeks at 25° C. or until a well-formed mycelial mat was produced, covering the surface of the liquid. At this time the mats were collected, freed from the remaining culture solution by filtering with suction, washed thoroughly with distilled water, and heated on a boiling water bath to destroy enzym activity. They were then placed over a hot-water radiator and allowed to dry at a temperature of about 50° C. When sufficiently free from moisture the mats were finely ground with a mortar and pestle and then placed in a desiccator over sulfuric acid until required for use. From 3 to 5 grams of fungus were taken for each analysis.

Both micro- and macrochemical methods were used. The former were of special value in following the progress of an extraction for the purpose of determining when it was complete and also for the detection of very minute quantities of cell-wall substance. The use of polarized light was also found to be a valuable aid. Had it not been employed the extremely minute quantity of cellulose present in fusaria hyphae would undoubtedly have been overlooked.

This investigation was undertaken with the assumption that the various cell-wall constituents such as cellulose, suberin, cutin, lignin, callose, etc., are entities and that their presence may be detected by certain definitely recognized and designated tests. Yet it frequently happens that one substance may completely cover another and thus prevent the penetration of reagents. In the spores and mycelium of *Cystopus candidus* the cellulose is completely covered with callose. In other cases the cellulose may be impregnated with fatty acids or with protein-like material. It is necessary to remove these before satisfactory tests can be obtained.

RESULTS

Some idea can be obtained from a preliminary examination of fusaria mycelium regarding its composition. Iodine and potassium iodid solution colors it yellow. When 70- to 75-percent sulfuric acid is then added,

the yellow color becomes brown or reddish brown. The same effect is produced with chloro-zinc-iodid (Artschwager, 1). When hydrolyzed with 5-percent sulfuric acid, dextrose is the only sugar which can be detected. This was later determined to come from glycogen within the cells. Anilin dyes stain cell contents only. Fresh hypha, after maceration for 48 hours in Javelle water, is completely disintegrated, leaving a copious, finely divided residue. This may indicate that the cell walls are cemented together or heavily impregnated with protein material, and that when this is removed they fall apart. This would be true if the walls were largely composed of chitin, because pure chitin itself is slowly disintegrated by alkaline oxidising reagents. Concentrated sulfuric acid in the cold produces marked alteration in the appearance of the mycelium, although the thalli remain intact yet very fragile. Under polarized light the hyphae are singly refractive. When treated with Sudan III, oil globules of the cell contents are brought more plainly into evidence but the cell walls remain uncolored. The action of the hyphae to ruthenium red is erratic. In young cultures, a week to 10 days old, the apical cells sometimes stain faintly red, while in old cultures, two to three months old, chlamydospores take the stain yet the rest of the mycelium remains uncolored. When oxidized with concentrated nitric acid and potassium chlorate a semi-liquid, oil-like substance collects upon the surface indicating the presence of fatty acids. Two furfural determinations, by Tollens' method, of 8-weeks-old cultures of *F. oxysporum*, gave 3.1 and 3.4 percent, respectively. No distinction was made between furfural and furfuroids. No methyl furfural was present. Chitin could readily be demonstrated as a constituent of the cell wall.

After repeated preliminary trials for the purpose of gaining an idea of the nature of the outer covering of the hyphae an intimate mixture of protein and pectic compounds was suspected. Three grams of dried and finely ground mycelium were treated with 50 cc. of 0.5-percent ammonium oxalate for one hour upon a boiling water bath. The solution was then filtered and two volumes of 96-percent alcohol added to the filtrate. An opalescent solution resulted. After standing over night a finely divided amorphous precipitate settled to the bottom of the flask. Similar results were obtained from four subsequent extractions with ammonium oxalate solution. Gradually decreasing amounts of precipitate were obtained in each case. After the sixth extraction the solution remained clear and no sediment collected.

The combined precipitates were filtered free from the liquid, washed with 75-percent alcohol and dried. This substance was found to be soluble in water, and dilute alkalis and responded to tests as follows:

Glyoxylic.....	faint reaction
Biuret.....	good reaction
Millon's.....	negative
Xanthoproteic.....	positive but not as good as the Biuret
Molisch's	good reaction
Furfural.....	good reaction
Phloroglucin-HCl.....	good reaction

After the ammonium oxalate extractions the hyphae appeared to present the same general characteristics in respect to dyes, iodine reagents, strong acids, and polarized light, as have been indicated in the preliminary investigations.

The residue was washed thoroughly with distilled water, several times with 96-percent alcohol, and dried. It was then digested for 24 hours in 100 cc. of an ammoniacal solution of cupric hydrate, sufficiently strong to dissolve cotton fiber or filter paper readily. In the preparation of the ammoniacal copper solution, the method outlined by Onslow (15) was followed. The ammoniacal copper solution was afterwards filtered off with suction through asbestos, diluted with several volumes of water, and the alkali neutralized with hydrochloric acid. A copious amorphous precipitate appeared, the greater part of which settled to the bottom, yet a portion remained in suspension. When filtered, washed free from acid with water and then with alcohol and dried, the precipitate gave the tests indicated below:

Biuret.....	good reaction
Xanthoproteic.....	good reaction
Glyoxylic.....	good reaction
Furfural.....	good reaction
Orcin-HCl-FeCl ₃	negative
Molisch's.....	negative
Phloroglucin-HCl.....	negative
Cellulose.....	negative

It is evident that the ammoniacal solution of cupric hydrate removed a protein material upon which the ammonium oxalate had no solvent action, and also that a different type of protein was obtained. The first was soluble in water whereas the latter was insoluble. Because of this difference in solubility it is reasonable to assume that the protein removed by the ammoniacal copper solution was probably held in combination in the cell walls, whereas that taken out by the ammonium oxalate came largely from the cell contents.

If the theory of the writer that the outer highly resistant covering of fusaria hyphae is a mixture of protein and pectic compounds is correct, it must necessarily follow that the pectic substance will remain in the residue, since it would be insoluble in an ammoniacal solution of cupric hydrate. It was no more possible to stain the mycelium with methylene blue and ruthenium red than in the preliminary trials.

The fungus was then treated with 5-percent acetic acid to remove the copper-oxid ammonia, washed free from acid, and again extracted with 0.5-percent ammonium oxalate for one hour upon a boiling water bath. When alcohol was added after filtration a gelatinous precipitate characteristic of pectic compounds was obtained. This gave positive orcinol, phloroglucin-HCl, and furfural reactions. Tests for proteins were negative.

An examination of the fungus residue under polarized light revealed it to be dimly doubly refractive. This characteristic indicated the presence of some refractive substance such as cellulose, suberin, cutin, or callose. The failure of the mycelium to fix the resorcin blue stain, and the persistence of the double refraction under polarized light after prolonged maceration in 1- to 2-percent aqueous potash, eliminated callose, at least as it was first characterized and reported by Mangin (5). Osmic acid produced a diffuse darkening of the hypha wall. Sudan III, cyanin, and alkannin showed no more tendency to stain the hyphae. This fact raised a doubt regarding the presence of suberin or cutin. It was realized, however, that in the case of cutin of the higher plants, the compact, outer layer does not take the fat stains as readily as the cuticularized layer underneath. Methylene blue or any of the other dyes which readily attack cellulose were of no avail.

Since the fatty acids which make up suberin and cutin are soluble in alcoholic potash, this course was decided upon next in order to eliminate completely the possibility of these substances being present. The fungus residue from the last ammonium oxalate extraction was washed first with water, then with alcohol,³ dried, and afterwards refluxed for one hour in 5-percent alcoholic potash. The solution first became yellow and then brown in color. After the alcoholic potash extract was separated by filtering, the sample was washed several times with boiling alcohol, and then the greater part of the alkali was removed by washing with water. No potassium phellonate could be detected in the alkaline alcoholic extract.

Examination under polarized light showed that the mycelium was more distinctly doubly refractive than it had been at any time previous, especially *en masse*. It fixed the methylene blue stain, coloring faintly blue, indicating that the layer which took the dye was very thin. With the iodine reagents, however, only a brownish color could be obtained. In spite of the fact that the test for cellulose could not be demonstrated at this time, the presumption that this substance was present was very strong. The residue was again extracted with 100 cc. of an ammoniacal solution of cupric hydrate for 24 hours. After filtering, diluting with water, and neutralizing with hydrochloric acid, a small quantity of precipitate very finely dispersed throughout the liquid separated out. Upon standing, this precipitate collected in small clumps and settled. This was filtered, and the sediment washed free from acid, collected on a watch glass, and dried. When treated with chloro-zinc-iodid, or with solutions of iodine and potassium iodid and 70-percent sulfuric acid, a brilliant blue color appeared, strongly suggesting cellulose.

In other analyses this precipitate was collected upon an asbestos filter, a known quantity of concentrated sulfuric acid added, and the mixture allowed to digest for 20 minutes. Sufficient water was afterward added to make a 10-percent acid solution. Hydrolysis was continued by refluxing for three hours. The product of hydrolysis was then filtered to remove the asbestos residue, neutralized with precipitated chalk, and evaporated to

small quantity. The yellow solution obtained reduced Fehling's and with phenylhydrazine and acetic acid formed osazones, which appeared in form and color identical with those obtained from dextrose. The quantity, however, was too small for further purification and melting-point determinations.

Examination of the mycelium after extraction with the ammoniacal solution of cupric hydrate revealed that it was still dimly doubly refractive in polarized light. Subsequent extractions yielded protein for the most part. Upon adding chloro-zinc-iodid to the precipitate the greater portion colored brown, yet an occasional speck of blue could be seen. It was thought probable that if further cellulose remained in the hyphae, as indicated by the double refraction, it might be so intimately mixed with protein material that the cellulose solvent could not readily penetrate, or at least acted very slowly. In order to facilitate this action, an attempt was made to break up the protein-cellulose complex by boiling the hyphae in 10-percent sodium hydrate solution for 20 minutes, then washing to remove the greater portion of the sodium hydroxid, and finally treating with concentrated ammonia. When examined in polarized light the mycelial mass was still doubly refractive, yet many of the individual hyphae appeared to have lost this characteristic. Small clumps of refractive material were seen separated from the hyphae. These gave a blue color when treated with chloro-zinc-iodid. The fungus residue was again extracted with ammoniacal cupric hydrate, and cellulose again demonstrated in the extract.

Examination of the mycelium after the last extraction with the cellulose solvent revealed that the doubly refractive property had disappeared. The entire mass was now dark. This fact, taken in connection with the other proofs advanced, is thought to be sufficient to establish beyond a doubt that cellulose is a normal constituent of the cell walls of fusaria mycelia. It is also evident that there is no reason to believe that the cellulose formed in this group of fungi is different from that of the higher plants. It is, however, found in different association. Instead of being mixed with lignin, as in the phanerogams, the cellulose of the fusaria is impregnated with fatty acids and protein material, and it is necessary first to remove these substances before its presence can be made manifest.

Because of the very minute quantity of cellulose present special care was taken to guard against error. The ammoniacal copper solution was always tested by neutralizing a portion of it before use. The asbestos used in filtering was previously extracted to remove any possibility of cellulose contamination. In a very large proportion of the analyses for the purpose of confirming the presence of cellulose, the fungus sample was first extracted with the cellulose solvent for the purpose of removing any lint which might have fallen from the cotton stopper of the culture flask and become incorporated with the culture. In none of these cases was an appreciable test for cellulose ever obtained.

After the removal of the cellulose the hyphae and septa were still found

to be intact. Attention was then directed to the specific tests for chitin. Iodine and potassium iodid solution still colored the mycelium yellow or slightly brown. When zinc chlorid or strong sulfuric acid was then added the color became deeper or even reddish brown. A portion of the mycelial residue was used for the chitosan reaction. It was boiled in concentrated potassium hydrate for 25 minutes, hardened in 96-percent alcohol, and then washed with water to remove the alkali. Upon adding a few drops of a solution of iodine and potassium iodid to the fragments of hyphae, a deep violet color instantly appeared. With other portions of the chitosan, tests were made for the formation of chitosan-sphaerites according to the method of Brunswick as given by Molisch (14). A small piece of chitosan about the size of a pin head was placed upon a glass slide under a cover glass. A few drops of 50-percent nitric acid were added and the preparation heated to boiling over a micro-burner. Upon cooling slowly, the sphaerites of chitosan formed. These could be readily discerned in polarized light by their characteristic black bands arranged in the form of a cross. After washing free from the acid the sphaerites could be stained with acid fuchsin or congo red.

The fungus residue was also found to be completely soluble in concentrated sulfuric acid, giving the acid solvent a brown color. Since no sediment remained it would appear that the basic material of fusaria hyphae, which forms the skeleton, is chitin. In this respect there is a marked contrast between fungi and the higher plants.

After extraction of the fungus with ammoniacal cupric hydrate solution followed by 0.5-percent ammonium oxalate, for the purpose of removing protein and pectic material, the dried residue was then extracted with anhydrous ether (redistilled from potash) for 3 hours. This treatment was considered sufficient to remove such uncombined oils or fats as might be present. The ether extraction yielded a light yellow oil. The different determinations were found to vary from 5.2 to 6.8 percent of the original weight of the samples. The iodine value of this oil, determined by Wijs' method, as outlined in Griffin (4), was 70.7, and the refractive index at 24° C. 1.485.

The fungus residue was then extracted with 5-percent alcoholic potash for 2 hours, making two extractions of one hour each with fresh solution. The filtered extract was first distilled to small volume, then evaporated upon a water bath to remove the alcohol. The residue was treated with warm water and found to be completely soluble. When cool, the soapy solution was acidified with sulfuric acid. The fatty acid, set free from the potassium salt, collected on the surface of the liquid. This was removed by extracting with ether, and the ether solution washed to remove the sulfuric acid and then evaporated. The amount of fatty acid was found to vary from 5.5 to 8.3 percent of the original weight of the fungus, depending upon the age of the culture. The iodine value of the fatty-acid content of a culture 2 weeks old was determined to be 88.9 and the refraction index at

40° C. 1.4765. There was indication that more than one fatty acid was present, but the small quantity of material precluded further examination. The oil and fatty-acid content of the hyphae was highest in young cultures, of about 10 days to two weeks growth. After autolysis had begun this value rapidly dropped. Cultures of *F. oxysporum* fourteen days old were used for the determination of oil and fatty-acid content.

DISCUSSION

These analyses make clearly evident the fact that the mycelium of the fusaria is complex in structure, undoubtedly much more so than former investigators realized. It is reasonable to assume that a similar complexity may be found to exist in other groups as well.

A knowledge of the composition of the hyphae enables one to understand better the remarkable resistance of the mycelium toward acids, and its inert characteristics toward dyes. While concentrated acids in the cold would probably destroy protein, the portions of the cell wall which were protected by the fatty acids would present the greatest resistance. Although little idea could be gained regarding the nature of the combination of the protein with pectic compounds, it is evident that the union was sufficiently close to satisfy molecular affinity and to render the mixture incapable of fixing stains. On the other hand it could be readily broken up by the action of an ammoniacal solution of cupric hydrate, of potassium or sodium hydrate, or Javelle water. The last reagent produced disintegration so completely that it was not deemed advisable to use it.

When strong oxidising reagents are employed the cellulose present in the hyphae may be overlooked owing to the fact that it may be destroyed before it is sufficiently separated from its associated linkages, and also because of the very small quantity of this substance. I am not aware that a cellulose fatty-acid combination has ever before been mentioned in connection with fungi.

Similar associations have been reported by Tupper-Carey and Priestley (17) in their investigation of the composition of the cell wall of the apical meristem of broad beans. The meristem tissue presented similar reactions toward dyes and strong acids as do fungus hyphae. The cellulose present could not be demonstrated until a cellulose-protein-pectin-fatty-acid complex had been broken up. The similarity of the two cases presents an interesting analogy between the mycelium of fungi and the apical meristem structure of higher plants.

SUMMARY

1. Analyses of the composition of the cell walls of twelve different species of *Fusarium* have been made and the same general structural plan has been found to hold for the group.

2. The outer covering of the hyphae was found to be a protein-pectic-compound, a cellulose-fatty-acid complex with a basic skeleton of chitin.

The relative quantity of cellulose was very small and the complex with which it was associated had to be broken up before it could be demonstrated.

3. The protein and pectic-compound part of the complex could be removed by the use of an ammoniacal solution of cupric hydrate and of 0.5-percent ammonium oxalate. The fatty acid extracted with hot alcoholic potash was found to have an iodine value of 88.9 and refractive index of 1.4765 at 40° C. In young, vigorous cultures the amount of fatty acid was found to be as high as 8.3 percent of the original dry weight of the mycelium while the free uncombined oils and fats constituted 6.8 percent.

4. It is suggested that the high oil and fatty-acid content in part accounts for the resistance of fusaria hyphae toward the action of strong acids, as well as other reagents.

5. No theory is advanced at this time regarding the probable origin and order of development of the cell wall. More detailed study is necessary in following closely the progress of growth before this can be done. More critical microchemical methods would greatly facilitate such an investigation.

6. The striking analogy between the structure of the apical meristem of broad bean and of the fusaria hyphae is pointed out.

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INTERNAL DECLINE (ENDOXEROSIS)¹ OF LEMONS VI. GUM FORMATION IN THE LEMON FRUIT AND ITS TWIG²

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INTRODUCTION

While this paper presents results of further studies on the physiological malady known as internal decline or endoxerosis of lemon fruits, it should be considered more especially as a direct continuation of paper V of the series (3) published under the same general heading.

In previous papers (1, 3) experiments were described the results of which showed (a) that in auxographic tests healthy lemon fruits began to contract one hour and twenty-five minutes earlier in the morning and to expand one hour and ten minutes earlier in the evening than endoxerotic lemon fruits, (b) that when healthy and endoxerotic fruits were detached from their twigs and potometers attached in their places, the twigs that had borne healthy fruits withdrew almost twice as much water from the potometers as did those that had borne endoxerotic fruits, (c) that when similar twigs were brought to the laboratory and successive segments of each twig tested as to their gas conduction capacity, it was found that the twigs that had borne the healthy fruits conducted more gas than those that had borne the endoxerotic fruits, (d) that the gas which was forced through the twig segments passed through the open vessels of the xylem and not through the parenchyma of the pith or bark, (e) that the diameter of the twig was not the important factor in governing these differences, and (f) that there were no tyloses in the vessels of the twigs that had borne either the healthy or the endoxerotic fruits.

The purpose of this paper is to describe and discuss the condition responsible for the differences mentioned in the preceding paragraph.

It has been reported in several papers of this series that the formation of gum in the lemon fruit, especially in the region of the vascular bundles, is one of the distinguishing characteristics of this malady. Therefore, after

¹ The term "endoxerosis," as previously suggested (1), is a technical name for this malady, and while it is so used in the text of this paper the term *internal decline* is retained in the title to facilitate reference to this series of papers. The term "endoxerosis" literally means internal drying, but gumming characteristically precedes the visible indication of drying, and the term as used in this paper is meant to include this characteristic.

² Paper No. 200, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

³ Paper No. V of this series appeared in the preceding issue of this JOURNAL.

the tests with the auxographs and the potometers had been made, twigs were brought to the laboratory to determine whether or not gum might be present also in the vessels of the twigs having borne the gumming fruits, thus producing a more or less effective barrier to the conduction of water. A casual examination did not reveal the presence of gum in the vessels of any of the twigs. The cross and longitudinal sections of both sets of twigs appeared to be healthy in all respects. Even when sections from several twigs that had borne endoxerotic fruits were made, stained, and examined under the microscope, no gum was detected. In order to determine whether a more extensive and careful microscopical examination would reveal any differences in the structure of the two sets of twigs, each segment of the twigs used in making the previously described gas-conduction tests was preserved in equal parts of glycerin and 95-percent alcohol. As soon as the gas tests were completed cross and longitudinal sections were made from each twig segment. These sections were stained and examined with the microscope.

The examination of these sections revealed the fact that while the conclusions drawn from the preliminary microscopical examinations were true for the particular twig parts examined they would not apply in general. In this later and extensive study gum was found to be present in the vessels in at least some parts of most of the pedicels or twigs that had borne endoxerotic fruits. The error in the conclusion from the preliminary observations was due to the chance choice of the portion of the twigs examined. It was found in the later study that where the lemon had been gumming for only a comparatively short time the gumming had not progressed farther back than into the button. In such cases as soon as the button was removed the gum-plugged vessels were removed also and the two sets of twigs conducted approximately equal amounts of gas. It happened that such twigs were examined in the preliminary tests and of course no gum was found. In some of the other cases the gumming did not extend back into the twig more than 1 to 2 centimeters. In the preliminary observations on such twigs the examination happened to be made back of the point to which the gum extended and, as a result, here again no gum was found.

METHODS

In the preliminary examination some of the fruit tissues were killed and fixed in medium or weak chrom-acetic acid solution. It was found, however, that with such treatment differential stains would not show a sharp contrast between the pectic substances of the middle lamella and the cellulose of the cell wall, hence the use of this solution was discontinued. These results substantiate the findings of Wood (14). At first the lemon fruit tissues to be studied were killed and fixed in Bouin's solution and imbedded in paraffin. It was later found, however, that fresh, unfixed material was best.

The buttons, pedicels, and twig segments which had been used in the gas-conduction experiment reported in the preceding paper of this series were

preserved for one to four weeks, while waiting to be sectioned, in equal parts of glycerin and 95-percent alcohol. In the later tests, because of fear that the presence of glycerin or alcohol in the tissues might lead to erroneous results when differential stains were used (14), the button, pedicel, and twig segments were sectioned without previous treatment.

The twig parts being not more than one year old, and often considerably less, no difficulty was experienced in making microtome sections of sufficient thinness. To facilitate holding the twig segments in place while sectioning, they were first imbedded but not infiltrated in paraffin on the end of a wooden block. In this manner sections only 6 to 8 μ thick could be made, although sections having a thickness of 15 to 20 μ were usually preferable for this study.

Single stains such as methylene blue, ruthenium red, magdala red, and Victoria blue proved helpful in studying the fruit tissues. Where a double stain was desirable safranin and light green were perhaps most satisfactory. It was more difficult to find a stain or combination of stains that would make the twig sections suitable for study. Several different stains or combinations were used for specific purposes; the ones most generally employed being methylene blue, ruthenium red, methyl violet, safranin, zinc chloroiodid, and methyl blue. The gum, cell contents (such as cytoplasm and starch grains), and certain changes which had taken place in the cell wall or middle lamella, were thus distinguished in at least a fairly satisfactory manner.

GUM IN FRUIT

Description and Location

In this malady gum does not usually begin to appear until the fruit is almost mature. In the majority of cases it first becomes evident while the fruit is still green, but in some cases its first appearance is after the fruit has become yellow. At first the gum is practically transparent, but in a short time the affected tissues and the accompanying gum assume a pinkish to light amber-brown color.

The vascular bundles in the peel of the styler end of the fruit are usually the place of origin of the first gum. From this point its formation may extend into the central core of the fruit and to isolated areas, forming "splotches," in any portion of the white inner portion of the peel (the albedo). In severe cases, the gumming may extend into the "pulp" (the carpels filled with juice sacs) at the styler end of the fruit from either the peel or the central core and cause one-third to one-half of the fruit pulp to lose its juice. The gum never breaks through or appears on the surface of the fruit. Macroscopically the gum as a rule appears to diffuse through the tissue, but in exceptional cases pockets $\frac{1}{2}$ to $\frac{3}{4}$ cm. in diameter may be found. With the aid of the microscope, however, it may be seen that there are many small pockets, especially in the region of the bundles.

The tissues first affected are the phloem and the bundle sheath, especially

the former. A case in which the phloem has begun to disintegrate is shown in a drawing of a cross section of a bundle in Plate XXXV at *f*, figure 3. A more advanced stage in which the phloem is practically entirely disintegrated is shown at *d*, figure 2. The gum formation and disintegration of tissues may extend into the bundle sheath (for initial stages see *e*, fig. 2, and *g*, fig. 3), and continue to progress until in places the xylem of the bundle may become entirely isolated from surrounding tissues. The parenchyma of the xylem in the bundles of the peel and central core may also be a source of gum production, but this does not appear to occur until much disintegration has occurred in the surrounding tissues. In severe cases, in which the larger gum pockets are formed, even the vessels themselves become at least partially disintegrated and segments⁴ of them may be found scattered promiscuously through the mass of gum. Gum may be formed also in the parenchyma of the albedo of the peel or of the core at points entirely isolated from the bundles (Pl. XXXV, fig. 5). These points which at first are isolated may later coalesce with each other or with the gumming areas of the vascular bundles.

As the gum is formed it imbibes much water and as a result pressure is produced. If a thin slice is cut from the peel of an actively gumming lemon, gum may be seen to exude almost instantly from the region of affected vascular bundles. Under this pressure a portion of the gum may be forced into the water-conducting vessels, where it partially or wholly plugs them. After the gum enters it apparently may be carried or forced in the vessel to a distance of at least several elements from its point of entrance. A study of the sections of the tissues shows that the gum is carried a greater distance toward the basal end of the fruit than toward the apical end.

Origin

One of the first symptoms of an abnormal condition in the fruit tissues may be indicated by what is apparently an increased granulation of the protoplasm. The first indication, however, of an actual disintegration is shown in the middle lamella, which begins to swell and fills the intercellular spaces (Pl. XXXV, fig. 5). The outer portion of the cell wall appears to be affected next, disintegration progressing inwardly until the whole wall may lose its identity (*h*, fig. 5). It is not uncommon to find cell walls completely broken down on one side, but intact on the other (fig. 5). Such cells are found on the margins of the pocket or in regions where gum formation is in the initial stages. In other cases the middle lamella on all sides of the cell may become dissolved, entirely isolating the cell from its neighbors. That such is the case may be demonstrated by placing in water on a slide a thin section of the tissue which contains a gum pocket. In the early stages of formation the gum is soluble in water and as a result the gum surrounding such cells goes into solution and the isolated cells may be seen floating in the central mass of gum solution.

⁴ Vessel elements, *i.e.*, the individual cells of which the vessel was originally composed.

There being no starch present in the lemon fruit at the time when the gum is being formed, except a limited amount in the chloroplasts at the surface of the peel while it is still green, it would seem that the middle lamella and the cellulose of the cell wall must be the principal if not the sole sources of the gum in the fruit.

GUM IN TWIGS

Description and Location

Endoxerotic gumming begins in the styler end of the fruit and advances progressively toward its basal end, then into the button (receptacle, calyx, and disc), into the pedicel, and finally into the twig itself. Therefore the amount of gum, or whether any gum at all will be found in the twig that has borne the endoxerotic fruit, will depend on the stage of advancement of the process of gum formation in the fruit. In no case has gum been found in a twig that had borne a healthy fruit, but all the twigs that had borne endoxerotic fruits have been found to contain gum, provided the stage of gum formation in the fruit is sufficiently advanced. That the gumming fruit is directly or indirectly responsible for the presence of gum in the twig is also indicated by the fact that where a healthy and an endoxerotic fruit are borne side by side on adjacent pedicels at the end of a twig, the xylem of the parts directly below the endoxerotic fruit will contain gum, but the half of the twig directly below the healthy fruit will be free from it. Such a condition is shown in Plate XXXVI, *m* and *n*, figure 7, where pedicel *n* bore the endoxerotic fruit. If both fruits on adjacent pedicels at the end of the twig are endoxerotic then gum will be found in both pedicels and in all of the inner xylem directly below the pedicels rather than below only one of them; the same holds true where, instead of two, only one endoxerotic fruit is borne at the end of a twig. Where the pedicel bearing the endoxerotic fruit is attached to the twig laterally instead of terminally, gum is found only in that portion of the xylem *below* (toward base of twig) and in direct communication with the pedicel; it is never found in the xylem *above* the point of attachment of the pedicel.

While the distance that the gum will extend back into a twig is governed by the extent to which gum has formed in the fruit which it bears, the distance will seldom, if ever, exceed 6 cm. By the time it has reached this distance the fruit has become so severely affected that an abscission layer is formed and the fruit drops. In the majority of the twigs examined the gum did not extend more than 1 to 3 cm. back of the fruit. The formation of gum in the twig does not continue after the fruit has been removed. It may be of interest to state here that as soon as the gumming fruit is removed not only does gum formation in the twig cease but processes soon begin which redissolve the gum and it disappears, presumably without having materially injured the twig, since this remains green and continues to grow in an apparently normal manner.

In some diseases of *Citrus*, gum may not only collect in certain tissues of the trunk or branch, but may come to the surface where it will form droplets or masses of varying size; or in other cases it may appear, apparently as the result of great pressure, in cylindrically spiral or ribbon-shaped projections. In all such cases the gum has its origin in the bark, in the cambium, or in the immediately adjacent xylem. In the case of the disorder under discussion, however, the gum is confined entirely to the xylem tissues of the twig, principally the older xylem, and the gum, as in the gumming fruit, never comes to the surface. It has been stated in a preceding paragraph that considerable pressure is produced in the lemon fruit in those regions in which gum is formed. From the behavior of the gum in the twig it seems probable that at least a certain amount of pressure is present there also, but such pressure as may be present does not cause any exudation from the cut surface.

Plate XXXVI, figure 6, shows in cross section the relative location of the gum in the xylem of a twig that had borne a fruit in the advanced stages of endoxerosis. The section represented here was taken from the twig, just back of the pedicel. In the upper portion of the button where the circles of isolated vascular bundles are more or less distinct the gum may be found in any of them, but, especially in the early stages, as one progresses downward through the pedicel and into the twig he finds that the gum is confined entirely to a comparatively narrow zone of xylem adjoining the pith. As the trouble becomes more severe in the fruit the circumference of the affected xylem area in the pedicel and twig increases until in the pedicel the gum may be found in all portions of the xylem from the pith practically out to but not in the cambium. In all of the hundreds of twig segments examined not a section was found which showed a deposit of gum in the cambium or cortex. In the twig, even in the most advanced stages, the gum-producing area does not usually extend outward from the pith more than half way to the cambium, except in an occasional isolated area.

Origin

The first conclusion as to the origin of the gum in the parts of the twig below the endoxerotic fruit was that it had been formed in the fruit and then transferred through the water vessel into the parts of the twig adjacent to the fruit. This conclusion had been reached because it had been noted that (a) the gum in the fruit was under considerable pressure, (b) no gum was found in the button, pedicel, or twig until a considerable amount was present in the fruit and when it did appear in these parts it appeared in them in the order mentioned, (c) when a healthy and an endoxerotic fruit were borne on adjacent pedicels at the end of a twig or where the pedicel bearing the endoxerotic fruit was attached at the side of the twig, gum was found only in that portion of the xylem of the twig directly *below* the endoxerotic lemon (Pl. XXXVI, *m* and *n*, fig. 7), and (d) there was no marked disintegration

of the xylem tissues of the twig parts such as there was in the fruit. However, when the sections of the twig parts were subjected to the proper differential stains it was found that there too, as in the fruit, certain tissues were undergoing change, and that at least the major portion, if not all, of the gum in these parts had been formed *in situ*, instead of having been transferred from the fruit.

An examination of the stained sections showed that the place of greatest activity was in the xylem parenchyma and medullary-ray cells adjoining the vessels. The portions of the walls of these cells that were on the side next to and touching the vessel took a different stain, or gave a different shade of color with a given stain, from the more distant portions of the walls. This was true also of the middle lamella. It was found that this changed condition of the wall of the cell adjacent to the vessel did not usually extend to the entire circumference of the cell, but in severe cases not only was the whole circumference affected but the wall of the second or even the third cell away from the vessel was found to be so. The walls principally affected appeared to be those lying parallel to the wall of the vessel.

The manner of passage of the gum from the surrounding xylem parenchyma and medullary-ray cells into the vessels was most readily determined from a study of the tangential longitudinal sections of the button, pedicel, and twig. In these sections as well as in the cross sections it could be seen that starch was apparently playing an important if not the major role as the source of the gum. This was indicated by the fact that in many instances the parenchyma and rays cells adjacent to a vessel, filled or partially filled with gum, were free of starch, while those farther away still contained an abundance of it. Reference should be made at this point, however, to Plate XXXV, figures 1 and 4; the former being a camera lucida drawing of a portion of a longitudinal section and the latter of a portion of a cross section of a twig a short distance back of the pedicel. In these sections is shown an abundance of gum in the vessels with little indication of starch depletion in the adjacent cells. This condition will be referred to later.

The middle lamella appeared to be affected first, then the cellulose wall, and finally the starch. That this was the order in which the different parts became affected was indicated by the fact that the middle lamella, and in many cases the cellulose wall also, gave a different stain reaction and droplets appeared on the inner wall of the vessel before there was any apparent diminution in the amount of starch in the affected cell. While the affected wall gave a different stain reaction than was given by the normal wall, in no case was the affected wall found to be completely or even noticeably decomposed, as was found to be true in the fruit tissues. It may be said here that the middle lamella also, even in later stages of gum formation, did not appear to be entirely disintegrated. These facts would seem to indicate that in the twig parts the starch was the source of at least much of the gum.

In the initial stages the gum collected in the pits between adjoining

cells; as more gum was formed it passed through the pits and collected in droplets opposite the pits on the inner face of the wall of the vessel (Pl. XXXVI, figs. 8 and 9). As the droplets increased in size they coalesced and finally, in many cases, not only was the element of the vessel completely filled but apparently a portion of the gum passed into adjoining elements of the same vessel or even into adjoining vessels (Pl. XXXV, *a*, *b*, and *c*, fig. 1, and Pl. XXXVI, fig. 10). Gum was also found in the affected medullary-ray and xylem-parenchyma cells which were in close proximity to but not necessarily touching a vessel.

DISCUSSION

Conduction of Water and Gas as Affected by Pressure of Gum

After finding that gum was present in greater or less amounts in the vessels of the twigs that had borne endoxerotic fruits it was not difficult to explain (*a*) why water could enter and be withdrawn from healthy fruits more easily than it could enter or be withdrawn from endoxerotic ones, (*b*) why the twigs that had borne healthy fruits withdrew more water from the potometers than those that had borne endoxerotic fruits, and (*c*) why more gas could be forced through twigs that had borne healthy fruits than through those that had borne endoxerotic ones. The variations in the three different series of tests were largely dependent upon the amount of gum in the vessels and the distance to which the gum extended back into the twigs. For the description and discussion of these tests see paper V of this series (3).

In the case of the auxographic tests which recorded the diurnal expansion and contraction of a given endoxerotic fruit, due to the entrance and exit of water (1), it seems that there can be little doubt that the gum in the vessels of the fruit itself, in addition to that in the vessels of the twig which bore it, had an influence upon the rate of entrance and exit of water.

Since in general the newly formed xylem conducts water more rapidly than that nearer the pith, and since, except in the more advanced stages, the gum is largely confined to the vessels of the inner half of the xylem, it is not so easy to explain why the twigs that had borne endoxerotic fruits withdrew approximately only one-half as much water from the potometers as the twigs that had borne healthy fruits. However, these results may be at least partially explained by considering that since these twigs were not more than from 9 to 12 months old, the inner xylem still retained a portion of its power to conduct water. That this was true was indicated by the fact that when similar healthy twigs were stood in a solution of acid fuchsin the dye ascended in all portions of the xylem. Examination of the twigs showed that the dye traveled in the inner half of the xylem about two-thirds as rapidly as it did in the xylem adjacent to the cambium.

The amount of gas which the twigs that had borne endoxerotic fruits conducted, compared with the amount they would have conducted had they been healthy, appeared to depend entirely upon the amount of gum in the vessels. Tests showed that no appreciable amount of the gas was conducted

through the cortex or pith (3), and tests with healthy twigs showed that all parts of the xylem were equally active in conducting the gas.

It is a well-known fact that under pathogenic or non-pathogenic conditions gum may be produced not only by many kinds of fruits, but also by the roots, stems, and branches of many kinds of plants. However, as far as is known to the writer, no case has been reported, where under either of such conditions the formation of gum in a fruit has been the direct or indirect cause of the presence of gum in the vessels of the twig on which it is borne.

Stimuli Possibly Causing Gum Formation

The stimulus responsible for the presence of gum in the lemon fruit and in the distal portion of the twig which bears it is not produced as the result of pathogenic conditions, and while it is not possible at this time to state definitely just what all of the non-pathogenic conditions are which are responsible for the particular kind of gum formation under discussion, yet two conditions may be mentioned which now appear to be important. The first condition or factor is that of temperature. The type of gumming under discussion does not appear in the lemon fruit and twig until the temperature has become comparatively high; whether this will be early or late spring depends on the season. The gumming may occur at any time from early or late spring until the average temperature becomes considerably lower in early or late fall. The amount of fruit and twig gumming is much greater in the inland districts than near the coast, which is in accord with the difference in temperature conditions in the two localities. The apparent effect of a relatively high temperature as a factor in gum formation in the lemon fruit and twig is in agreement with the findings of Higgins (10) on the effect of different temperatures on gum formation in various kinds of twigs, such as the peach, plum, and cherry. Swarbrick (13) found in his experiments on the formation of gum in the cut but still attached stems of sycamore, rhododendron, plum, and apple that the production of gum was decidedly more rapid during the months of May to August inclusive, during the period when growth and sap movement were most active. This is also the period of highest temperature during the year, and in the light of this fact and of previous results it does not seem impossible that the rise in temperature may also have been a factor.

The second factor which appears to be very important in causing the formation of gum in the lemon fruit and its twig, and one that is closely related to if not directly caused by the first factor mentioned, is that of a water deficit in the tissues concerned. Perhaps it may be well to call attention to the fact that this condition does not necessarily predicate a lack of moisture in the soil. In the case of *Citrus* there may be a marked water deficit in the fruit tissues for many successive days, even though the amount of moisture in the soil be well above the wilting coefficient. Many cases have been found where such a deficit has existed in the fruit both day and

night, over periods of two weeks or more (1). Such deficits are caused by the fact that the roots, under the existing climatic conditions, are not able to supply a sufficient amount of water to counteract the loss through excessive transpiration, and hence not only is the fruit deprived of its share of the absorbed water, but a portion of the water which entered the fruit during the night is withdrawn during the day. Therefore it does not seem improbable that such water deficits may be one of the important factors which cause gum to form in the tissues. Butler (4), in studying the causes of other forms of gumming in *Citrus*, came to the conclusion that the presence of an abundance of water in the tissues was necessary, but that does not appear to be the case in endoxerosis. On the other hand, Higgins (10) found that a water deficit in the tissues very decidedly stimulated gum formation. In the fruit the first parts to be affected are usually the bundles in the styler end of the peel, the parts most distant from the source of water supply.

That a water deficit in the twig, as well as in the fruit, appears to stimulate the formation of gum in endoxerosis is further indicated by the fact that the gum first appears in that portion of the twig xylem least concerned with water conduction, namely, the older and less active portion which lies near the pith. As the fruit matures the xylem of the pedicel becomes progressively less active in water conduction, and not long after the fruit drops or is picked the pedicel ceases to function and becomes detached from the twig. It was interesting to note that as the xylem of the pedicel became decreasingly active, gum became progressively evident toward the cambium. In the early stages gum was found only in the inner portion of the pedicel xylem, but just about the time the endoxerotic fruits were ready to drop, it appeared in vessels in all portions of this tissue. This condition appears to confirm the conclusion that a water deficit in the xylem of the twig acted as a stimulus to gum formation; the less active the xylem became, the more the tissues were exposed to a water deficit.

At least to the extent that it initially begins to form near the pith and gradually spreads outward, the type of gumming in endoxerosis is more nearly comparable to the formation of gum in heart wood, as described by Coster (5) and others. This again would indicate that at least partial desiccation may be an important factor in stimulating the formation of gum.

Unpublished results obtained by Reed and Bartholomew in a study on wind injury of *Citrus* also appear to indicate that a water deficit may act as a stimulus to the formation of gum in the tissues concerned. During a strong desiccating wind, accompanied by a comparatively high temperature, the mature leaves on the windward side of orange trees may be killed so quickly that there is no time for an abscission layer to form, and as a result these particular leaves may remain attached to the twigs for several weeks. In the course of one to two weeks gum may not only be found in the vessels of the portion of the twig bearing the dead leaves, but it may extend several centimeters downward into the portion of the twig bearing uninjured leaves.

In the portion of the twig below the zone of injured leaves the gum is confined to the inner portion of the xylem as it is in the early stages of endoxerosis. It does not seem improbable that excessive evaporation here, as in other cases, may have been one of the important factors causing gum formation. At any rate the two conditions, an abundance of water and active growth, stated by Butler (4) to be necessary for the formation of gum in the diseases of *Citrus* and *Prunus* which he studied, were certainly not present.

Gum Formation and Growth Activity

The endoxerotic gumming of the lemon fruit and twig is not necessarily associated with growth activity as has been found to be true for some types of gumming. Gum does not appear in the lemon fruits and pedicels until growth has practically if not entirely ceased. In the case of the twigs to which the gumming pedicels were attached, gum was found only in those portions of the xylem in which growth was least active, principally in the inner half.

Tissues and Substances Concerned in Gum Formation

That the type of gumming which is found in endoxerosis is different from the types usually encountered in *Citrus* and other fruit trees is indicated by the fact that in the latter cases the gumming occurs in the cortex, cambium, or newly formed xylem. In the studies of Fawcett (6, 7) and Butler (4) on *Citrus* gummosis, and even in the experiments of Swarbrick (13), Higgins (10), and others, where the entire end of the cut branch or twig was exposed, it was found that the source of initial and most active gum formation was in the region of the cambium rather than near the pith.

There has been much discussion as to whether in certain cases of gumming it was the pectic substances and cellulose or the starch or other carbohydrate materials which were the principal sources of gum. The case in hand is interesting in this connection. In the case of the lemon fruit no starch is present and consequently the gum must have come from some other source. From the fact that the middle lamella and the cell wall were both found to be partially or wholly decomposed it would seem that they must have been the source of the gum. Such cell contents as mono- and disaccharids may have been factors, of course, but the results of previous chemical tests on endoxerotic tissues indicate that they did not play an important, or at least not the major, part in this connection (2). In these chemical tests it was found that the albedo of the peels of gumming lemon fruits contained from 39 to 63 percent more pentosans than did similar tissues from the healthy fruits, and that these pentosans were formed at the expense of hexosans rather than mono- and disaccharids.

In contrast to the condition in the fruit, where no starch is available for gum formation, there is an abundance of starch in the twig parts, and it appears that it or its decomposition products may have been a source of

much of the gum. It is possible that the disappearance of the starch may have been simultaneous with, though not directly related to, the appearance of gum, but this does not seem probable when it is considered (a) that as the starch disappeared globules appeared in these cells which gave the same or nearly the same staining reaction as did the decomposition products of the middle lamella of the fruit, and (b) that the amount of gum formed was probably much more than could be accounted for by the relatively small number of middle lamellae and cell walls affected. The fact that in the affected xylem of the twig parts neither the cell wall nor the middle lamella appeared to be completely destroyed may be explained by the results of recent works such as those of Rhoads (11), Ritter (12), and Harlow (9) in which it was found that in secondary xylem not only the cell wall but the middle lamella also may contain much lignin. Haberlandt also states that "in the case of lignified and suberised tissues the middle lamellae are often more or less strongly lignified." Ritter in working with the red alder and the western white pine found a much higher percentage of lignin in the middle lamella than in the cell wall. Although the middle lamella and the cell wall in the xylem of the lemon twig did not completely disintegrate it was apparent that they were changed and that this change at least began before there was any distinguishable diminution in the amount of starch within the cells. The changed condition first appeared and was most evident on the side of the cell touching or near to the vessel.

In no case in the twig parts were gum pockets formed, and in the twig itself the destruction was not sufficient to cause the death of the tissues involved. At least the twig could continue to grow indefinitely after the fruit had dropped or had been removed, and appeared to be in a healthy condition.

Transfer of Gum from Seat of Origin

Whether all of the gum found in the xylem portions of the twig back of the gumming fruit was formed *in situ* or whether at least a portion of it was transferred from the fruit to the twig proved to be a puzzling problem. That a large portion of it was formed in the twig itself cannot be questioned and it is possible that all of it may have been formed there. However, in observing such conditions as are illustrated in Plate XXXV, figures 1 and 4, and Plate XXXVI, figures 9 and 10 it does not seem impossible that the gum, or the decomposition products which later formed the gum, may have traveled in the vessels some distance from the point of their entry into them. The condition illustrated in the second set of figures mentioned clearly indicates that the gum in the vessels is being formed from the neighboring cells. This is especially true for figure 9, but in the case of figure 10 the evidence is not quite as clear since much starch still remained in the cells adjoining the vessel, as is true for the first two figures mentioned above. Either the gum in these vessels came from adjoining cells, which could not be detected in serial sections, or it migrated into them from some point

where gum was being actively formed. Such a condition as is illustrated in these two figures is sometimes found at the extreme point to which the gum extends back into the twig. Such a condition occurs more often in the vessels of the gumming fruit. Here it is not uncommon to find gum in the vessels a centimeter or more beyond the point where stains or the visible decomposition of the tissues indicate that gum is being actively formed. In this connection it may be mentioned again that the gum in the fruit was under considerable pressure. However, it must be stated that it was never possible to trace gum continuously in a given individual vessel from any portion of the fruit back into the xylem of the button, except from near the base of the fruit where it was joined to the button. This would tend to discredit the idea that the gum in the twig parts had been transferred to them from the fruit unless one were to consider that certain decomposition products had been carried in liquid form from the fruit back into the vessels of the twig, where they were later changed into gum. While this does not seem an impossibility yet it does not appear to be a probability. It should be remembered that the conditions mentioned in this paragraph, *i.e.*, where gum was found in the vessels at some distance from its apparent seat of origin, were the exceptions rather than the rule.

Staining Reaction of Gum

As was found to be true especially by Butler (4), Fawcett (6, 7), and Swarbrick (13) in their studies on gumming, the gum in the endoxerotic fruit and its twig gave different staining and solubility reactions, apparently depending on the degree of chemical transformation. In some cases it was found that even after gum had been accumulating in the vessels for a week or more it might be almost entirely dissolved by placing the tissue sections in water over night. After a longer time, however, the gum became insoluble in water, apparently becoming of the nature of wound gum. The gum did not usually take the lignin stain until at least a short time after it had collected *en masse* in the lumen of the vessel. As it migrated through the pits and formed in droplets on the inner surface of the walls of the vessels it usually took the cellulose stain. In some cases the droplets took that for lignin but it seems probable that in such cases activity had previously ceased and they had become of the same chemical nature as the larger and older masses.

In the description and discussion the term "gum" has in general been applied alike to all of those stainable decomposition products located in or between the xylem-parenchyma and ray cells or which had migrated from these places into the vessel lumen. However, it is realized from the very nature of the materials and processes concerned that many intervening substances must have been formed before starch, cellulose, or peptic substances could have been transformed into water-soluble or insoluble gums. The stain reactions varied all the way from different shades with

pectin or cellulose stains to cases where the substance took only a lignin stain. The variations in staining reactions were apparently dependent upon the age of the substance, or perhaps, more correctly, upon the number or nature of the chemical changes which had occurred in it.

SUMMARY

1. The presence of gum in the vessels of endoxerotic lemon fruits, and in the vessels at the distal end of the twigs on which they were borne, materially retarded the rate of entrance and exit of water into and from the fruits, the rate at which the twigs withdrew water from potometers, and the rate at which gas could be forced through the twigs.

2. In the buttons where the bundles had not yet joined to form a continuous zone the gum did not appear to be restricted to any particular bundles; in the pedicel the gum was at first confined to the portion of the xylem bordering the pith, but in the final stages it might be found in any of the vessels practically out to the cambium; in the twig the gum, even in the most severe cases, was confined almost entirely to the inner half of the xylem.

3. Gum ceased to form in the twig after the gumming fruit had been detached from it, and the twig continued to grow, apparently not having been materially injured by the formation and the presence of the gum.

4. The distance to which gum was found to extend back into the twig parts appeared to depend upon the amount of gumming in the fruit, but even in the most severe cases it was not found to go back more than 6 centimeters.

5. If a gumming lemon had been borne singly at the end of a twig gum was found in any portion of the inner xylem of the twig, while if healthy and gumming fruits had been borne on adjacent pedicels at the end of a single twig or if the gumming fruit had been borne on the side of a twig, then gum was found only in that portion of the xylem directly below the gumming fruit.

6. The middle lamella and the cellulose wall appear to have been the principal source of the gum in the fruit. The phloem appears to have disintegrated first, then the bundle sheath, and finally the parenchyma between the bundles. In many cases disintegration proceeded until the tissues concerned were totally destroyed and gum pockets were formed.

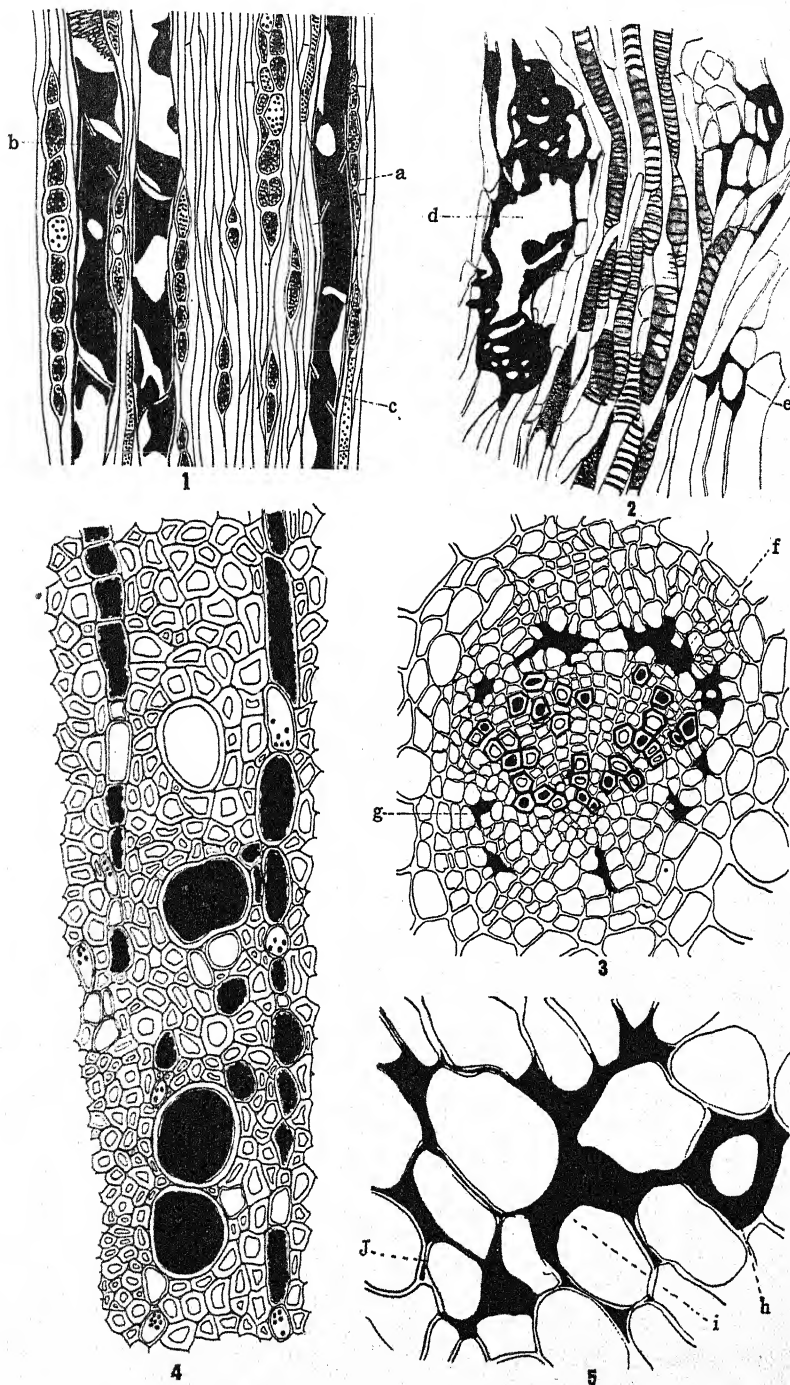
7. In the twig parts starch appears to have been the principal source of the gum, but the middle lamella and apparently the cell wall were also affected, especially the former. In no case in the twig were gum pockets found, the severity of gumming being determined by the presence of much or little gum in the lumen of the vessels. As the disintegration proceeded the decomposition products passed through the pits in the cell walls and collected in droplets on the inner face of the vessel wall. As the gum increased in amount these droplets coalesced and partially or wholly filled the vessel segment.

8. A very large part of the viscous substance which has been called gum remained water-soluble for some time, in some cases at least a week or ten days, after it had migrated into the vessels. Later, however, it became less soluble and finally became insoluble in water, in which state it should probably be referred to as wound gum.

During the continuation of the study of this problem of gumming in the lemon fruit and twig it is hoped that not only further evidence as to the origin of the gum in the different tissues, but also some evidence as to the nature of the stimulus which induces the gumming, may be presented. With reference to the latter it may be said now that even though the stimulus to gumming may first affect the fruit, which in turn transmits the stimulus to the twig parts, yet when the fruit is removed from the twig it no longer continues to form gum, and gum formation also ceases in the twig.

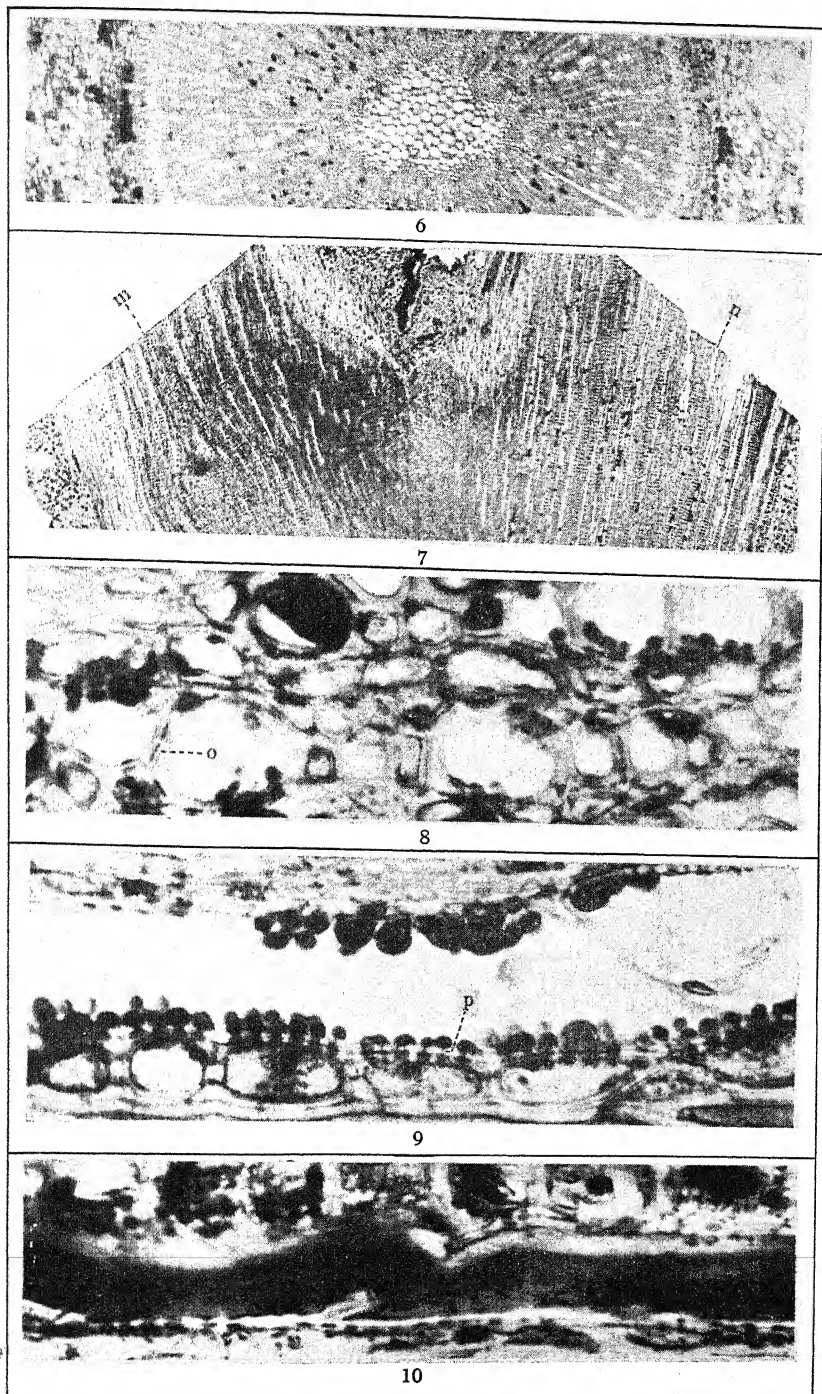
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BARTHOLOMEW: INTERNAL DECLINE





EXPLANATION OF PLATES

PLATE XXXV

FIG. 1. Tangential longitudinal section of a lemon twig that had borne an endoxerotic fruit; drawing taken from a section of the twig showing the lower extremity of gum-invaded vessels. Note the abundance of gum in vessels, the comparative abundance of starch remaining in the adjoining ray and xylem-parenchyma cells, at *a*, *b*, and *c*, and the manner in which the gum may pass from one vessel element to another (see also photograph of same in Pl. XXXVI, fig. 10).

FIG. 2. Longitudinal section of a vascular bundle from a gumming area in the peel of an endoxerotic fruit. Note the formation of gum in the bundle sheath at *e* and the complete breaking down of the phloem at *d*.

FIG. 3. Same as figure 2 except a cross section. Note gum formation in phloem at *f* and in bundle sheath at *g*.

FIG. 4. Cross section of a lemon twig that had borne an endoxerotic fruit; conditions similar to those indicated in figure 1.

FIG. 5. Section from a gumming area in the parenchyma of the peel of an endoxerotic fruit. Note condition of middle lamella at *j*, the disappearance of the wall on one side of the cell at *i*, and the complete disappearance of the cell wall at *h*.

PLATE XXXVI

FIG. 6. Cross section of a lemon twig that had borne an endoxerotic fruit. Note that the gum is largely confined to the inner half of the xylem. The twig was probably not over ten months old, the annular appearance in the xylem being due to different growth cycles of the first season.

FIG. 7. Longitudinal section of a lemon twig that had borne a healthy and an endoxerotic fruit on adjacent pedicels; the healthy fruit at *m* and the endoxerotic one at *n*. Note gum in vessels of twig at *n* and none in vessels at *m*.

FIG. 8. Cross section of gumming lemon pedicel showing gum migrating into vessels from adjoining ray and xylem-parenchyma cells. Note condition of middle lamella at such places as *o*; also practically complete absence of starch in the cells adjoining the vessels.

FIG. 9. Portion of a single vessel and immediately adjacent cells as seen in a tangential longitudinal section of a lemon pedicel that had borne an endoxerotic fruit. The gum on the upper side of the vessel came from adjoining xylem parenchyma while that on the lower side came from medullary-ray cells. As indicated in the figure, both sets of these cells were practically free of starch. Note migration of gum through the pits, as indicated at *p*, and similar places.

FIG. 10. Same as figure 4 except that it represents a condition found at the lower extremity of gum-invaded vessels in the twig. The vessel elements are filled with gum and while there is evidence that at least some gum has migrated into the vessel from neighboring ray and xylem-parenchyma cells, yet these cells still contain much starch. Note gum connection through the opening in the cross wall between the two vessel elements (see also *a*, *b*, and *c*, Pl. XXXV, fig. 1).



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STUDIES ON CALLUS TISSUE

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INTRODUCTION

A series of intergeneric and interspecific graft unions were made between members of the Solanaceae for the purpose of studying the possibility of antibody production. The physiological and morphological changes in the graft unions treated from a purely immunological point of view will be considered elsewhere. The present publication deals with certain macroscopical and microscopical observations on the callus.

METHODS

Grafting

All of the methods of grafting described by Winkler (1924) were tried, but most frequently whip grafting, tongue whip grafting, cleft grafting, saddle grafting, and budding. The best results were obtained by simple whip grafting. The scion and the stock were bound together with ordinary cotton twine 0.5 to 1.5 mm. thick. No wax was used after binding. Successful unions were obtained in about 90 percent of the cases. The callus wounds healed in from 5 to 10 days after grafting, depending on the environmental conditions, the age of the graft components, and the specific regenerative potency of the scion and stock.

The present work covers only the observations on the callus after simple whip grafting.

Cytological Methods

The following modification of Bouin's solution was used for fixing callus tissues: 74 percent saturated solution of picric acid, 20 percent commercial formaldehyd, 5 percent glacial acetic acid, and 1 percent lactic acid. To this mixture of solutions, 2 gm. chromic acid and 2 gm. urea were added per 100 cc. The fixative was used 24 hours after its preparation.

In order to make sections suitable for study, shoots of graft unions were cut just below the callus, and immersed immediately in the above-mentioned fixative to a point above the callus. The transpiration of the shoot leaves facilitated the penetration of the fixative in the callus. Calluses less than

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10 mm. in diameter were separated from the shoots after keeping them 12 hours in the fixative; larger ones were left 24 hours. By this time, the fixative had reached the middle of the shoots and penetrated the pith. After having been separated from the shoots, the calluses were left 12 hours longer in the fixative. After washing and dehydrating—the duration of these processes depending upon the size of the pieces used—the soft calluses were imbedded in paraffin, and the hard, woody ones in celloidin. Sections 8 to 16 μ thick (1.5 to 2.5 cm. long and 1.0 to 1.5 cm. broad) were made through the entire piece. The sections were treated with the following stains: safranin, Heidenhain's iron-alum haematoxylin, Heidenhain's iron-alum haematoxylin and safranin, gentian violet and anilin water solution, iodine, and safranin anilin water solution and brilliant green. The union of the components, the distribution of the starch in the callus, the secondary thickening of the single pith cells or groups of pith cells of both sides of the callus, and the bacterial nests in the callus were investigated.

MACROSCOPICAL OBSERVATIONS ON THE CALLUS

In almost all intergeneric graft unions it was necessary, in order to obtain satisfactory growth of the scion, to maintain an approximate equality between the amount of green mass (leaves and stems) of the scion and the stock. As soon as either scion or stock predominates, it kills or injures the opposed tissue. This occurred most frequently when the stock predominated. The phenomenon was not observed very frequently, however, in graft unions between species of the same genus.

The calluses of the intergeneric, and of certain interspecific, graft unions produce various types of tumoral formations. These formations remind one of the crown galls studied, and even artificially produced, by Smith (1911, 1912, 1917, 1923) and Riker (1927). Plate XXXVII, figure 1, shows the callus of *Nicotiana rustica* grafted on *Datura ferox*, and Plate XXXVIII, figures 7, 8, and 9, the calluses of *Nicotiana Langsdorffii* grafted on *Solanum nigrum*. The tumoral tissues in these cases are chiefly produced by the scion. Similar phenomena were observed in the following graft unions: *Nicotiana Sanderae* grafted on *Datura Wrightii*, *Solanum nigrum* grafted on *Datura ferox*, *Solanum nigrum* grafted on *Nicotiana Tabacum*, *Lycium Barbarum* grafted on *Solanum Lycopersicum*, *Salpiglossis sinuata* grafted on *Datura ferox*, etc.

Figure 2 shows another tumoral formation. This callus is from *Nicotiana Sanderae* grafted on *Datura ferox*. The tissues of the scion (*Nicotiana Sanderae*) form numerous leafy shoots immediately above the callus. Smith (1917) has produced this type of tumoral formation in various plants by bacterial inoculation (*Bacterium tumefaciens*), and has called it atypical or embrional teratoid tumor.

Solanum Lycopersicum grafted on *Datura Wrightii* (Pl. XXXVIII, figs. 5 and 6) forms numerous small disorganized tumors for a distance of about

25 to 35 cm. above the callus. Near the callus the tumors are large and numerous. Their number and size steadily diminish from the callus upward.

Another type of tumoral formation is a root production by the scion, exhibited immediately above the callus. This type is also solely a scion formation, as are the other types mentioned above. Smith (1923, fig. 6) has reported a similar formation caused by bacteria. I have observed root formations of the scion just above the callus in the following graft unions: *Nicotiana Langsdorffii* grafted on *Nicotiana rustica*, *Nicotiana Tabacum* grafted on *Datura Wrightii*, *Solanum nigrum* grafted on *Nicotiana Sanderae* (fig. 4) and on *Datura ferox*, etc. The roots usually began to appear in from 20 to 30 days after the grafts were made.

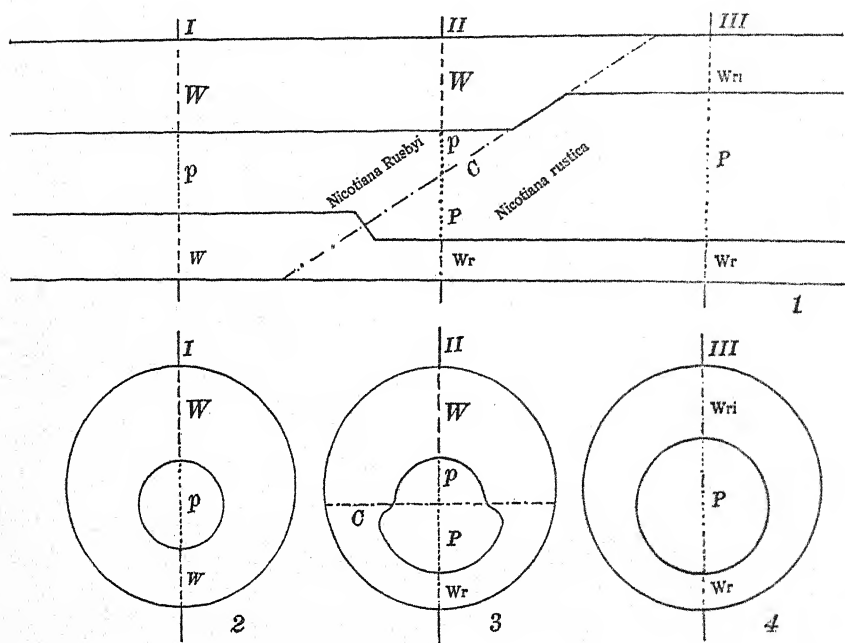
The green color of the stock in the graft union *Solanum nigrum* on *Nicotiana Sanderae* (fig. 4) changes in about 20 days to yellow, then fades; and in from 50 to 70 days, the stock (*Nicotiana Sanderae*) begins to die. The necrosis starts at the point where the lowest part of the callus ends, and in a few days (10 to 15) reaches downward a distance of about 10 to 15 cm. During this period, the scion still lives. It is supplied with food coming from the roots by the still living stock tissues on the side opposite the dead tissues (left side in fig. 4). The necrosis proceeds faster in the direction of the vessels than in a horizontal direction. The last bit of tissue to die is that which is in touch with the highest point of the scion (left side in fig. 4). The picture of the union in figure 4 was taken when total necrosis of the stock was approached.

A similar phenomenon is presented by the graft union *Nicotiana glauca* on *Capsicum pyramidale* (fig. 3). The green color of the scion (*Nicotiana glauca*) in a small area of 3 to 4 mm. around the callus changes to pale yellowish (a) in about 20 to 30 days. Paraffin sections of such a callus show agglutination of the plastids in the pale yellowish area.

In the graft unions *Nicotiana Sanderae* (stem with violet pigment) on *Datura Wrightii* (green stem), *Solanum nigrum* (stem with violet pigment) on *Nicotiana alata* (green stem), *Datura ferox* (with violet stem) on *Nicotiana rustica* (with green stem), etc., the violet pigment of the scions continues during the life of the unions only in the scion tissue, without any effect on the color of the stock tissue. The pigmentation of the scions was greatest just above the callus. The same phenomenon has been observed when the violet pigment was present in the stock and not in the scion. Such unions were *Nicotiana rustica* grafted on *Datura ferox* and *Nicotiana Tabacum* grafted on *Solanum nigrum*. The violet pigment present in one species was not able to affect the tissues of the opposed species during the life of the unions.

When a woody plant is grafted on an herbaceous plant by simple whip grafting, a change in the ratio of wood and pith occurs around the callus, and the wood is asymmetrically distributed above and below the callus in transverse sections. This phenomenon, schematically represented in text

figures 1 to 4, was observed in the graft union *Nicotiana Rusbyi* on *Nicotiana rustica*. Text figure 1 represents a longitudinal section of the callus of the latter union. At the left of the callus (*C*) is the scion (*Nicotiana Rusbyi*); at



TEXT FIG. 1. Longitudinal section through a 5-months-old callus of the graft union of *Nicotiana Rusbyi* grafted on *N. rustica*. FIG. 2. Transverse section through I of text figure 1. FIG. 3. Transverse section through II. FIG. 4. Transverse section through III. *C*, callus; *W*, wood of *Nicotiana Rusbyi* component; *P*, pith of *N. Rusbyi* component; *Wr* and *Wri*, wood of *N. rustica* component; *P*, pith of *N. rustica* component.

the right is the stock (*Nicotiana rustica*). A transverse section through I of text figure 1 is given in text figure 2; through II, in text figure 3; and through III, in text figure 4. The pith of *Nicotiana Rusbyi* in the callus is slightly enlarged; that of *Nicotiana rustica*, somewhat suppressed. In both components, the wood near the callus is thicker on the lower side of the callus. This asymmetrical distribution of the wood around the callus is the cause for the excentric position of the pith.

MICROSCOPICAL OBSERVATIONS ON THE CALLUS

Göppert (1874) called the joining tissue between scion and stock "intermediäres Gewebe," and described it as a product of the medullary rays. Sorauer (1875) questioned this as follows: "... trotz der Aehnlichkeit des intermediären Gewebes mit den normalen Markstrahlzellen, das erstere

doch nicht ein Product der letzteren ist. Vielmehr sieht man, dass alle zur Bildung eines normalen Holzkörpers ursprünglich angelegten cambialen Zellen, also sowohl die zur Verlängerung der Markstrahlen als auch die Holzzellen und Gefäßen bestimmten, sich an der Bildung des intermediären Gewebes betheiligen." And he writes further that ". . . der Zwischenraum . . . zwischen Edelreis und Wildstamm . . . durch intermediäre oder Kittgewebe (Sorauer's term for joining tissue) ausgefüllt ist." He does not give any figures substantiating his observations.

In studying the longitudinal sections of the callus, I have found that the joining tissue between stock and scion is usually stock product in both the pith and the wood zones. Thus, in Plate XXXIX, figure 10, and Plate XL, figure 18, are given sections of the callus of the union *Solanum nigrum* (n) grafted on *Nicotiana rustica* (r), which show that the cells of the stock produce the joining tissue by division in the direction of the callus (C). The sections are made from material fixed 4 months after grafting. Figure 11 gives an even better illustration of this phenomenon. It represents the callus of the union *Nicotiana Tabacum* var. *macrophylla* (M) on *Solanum Lycopersicum* (L), 15 days after grafting. The scion manifested no production of joining cells, while the stock has produced a joining tissue (j) of about 8 to 12 cell layers.

The same phenomenon was observed not only when the graft components belonged to different genera, but also when they belonged to the same genus. Thus, longitudinal sections of the graft union *Nicotiana rustica* on *Nicotiana glauca* are given in figure 16 and in figure 19, the callus being fixed 50 days after grafting. The polarity (direction of the cell rows) of the joining tissues in both intergeneric and interspecific graft unions diverges from the polarity of the scion and stock tissues. The joining tissues (j) keep always a direction approximately perpendicular to the callus plane. Their cells are usually smaller than the old cells of the scions and of the stocks.

In the woody region of the callus, the joining tissue (j) is also derived from the stock tissue and is directed toward the callus plane. Sections of the woody region of the graft union *Nicotiana Rusbyi* on *Nicotiana rustica* from material fixed 5 months after grafting are shown in figures 21 and 22.

The cells injured during the graft operation in the callus gradually disappear during the time of growth together of both components. In the young calluses (figs. 11, 12, 13, 14, 16, 19, 23, and 25) this injured tissue appears in the slides as a small zone composed of cell walls and disintegrated cell elements which stain very deeply with all dyes tested. In the old calluses (figs. 10, 18) this remnant appears in the sections only as a slight stained line, the callus line; and even the latter is no longer present in the wood of the graft union *Nicotiana Rusbyi* on *Nicotiana rustica* (figs. 21 and 22). Here the callus is represented only by the divergent polarity of the joining tissue produced by the stock. The callus line disappears first in the region of the cambium and vessels (figs. 13, 16, and 18) and finally in the

region of the pith (fig. 20). Figures 12 and 13 are sections through a 15-days-old callus of the graft union *Nicotiana Tabacum* var. *macrophylla* on *Solanum Lycopersicum* to show the cambium zone. The photomicrograph in figure 12 is taken from the highest joining region of the callus of a simple whip graft, while that in figure 13 is taken from the lowest joining region of the same callus. It is obvious from these two figures that the cambial connection appears first in the lowest region of the callus. This apparently causes the interruption of the callus line (C) in figure 13. Figure 14 (*Solanum Lycopersicum* grafted on *Nicotiana glauca*), figure 16 (*Nicotiana rustica* grafted on *Nicotiana glauca*), and figure 18 (*Solanum nigrum* grafted on *Nicotiana rustica*) show the same discontinuity of the callus line (C).

Sections through the tumorous formation of the callus (fig. 17) show a multipolar arrangement of the proliferated tissue. Here and there in the proliferated tissue can be seen small abnormal vascular bundles (v), either single or in groups. Abnormal vascular bundles are also formed very frequently in the joining tissue of the callus (figs. 14, 15, and 16). They are optically active in polarized light, and appear very distinctly in preparations stained with safranin anilin water solution and brilliant green in which the cells with secondary thickening are stained green, while all others are stained red.

An accumulation of starch in the scion tissues above the callus (fig. 23) was observed in many graft unions. When *Solanum tuberosum* is grafted on *Nicotiana rustica* the accumulation of starch above the callus is so abundant that the potato forms aerial tubers near the callus.

Cells (fig. 24) containing sand and groups of pyramidal crystals of calcium oxalate (determined after Molisch, 1921) appear very frequently around the callus in both components. Their frequency diminishes with the distance from the callus.

In a 15-days-old callus (*Nicotiana Tabacum* var. *macrophylla* on *Solanum Lycopersicum*) several bacterial nests were found just between the two components (fig. 25). No bacteria were found in the calluses of older graft unions.

DISCUSSION

The joining tissue in a graft is chiefly stock product, apparently because of insufficient water supply in the scion during the first few days after grafting. After the two components have grown together, however, the growth capacity of the scion above the callus is greater than that of the stock.

The scions of intergeneric graft unions and those of certain interspecific graft unions form various types of tumors, aerial roots, and leafy shoots just above the callus. Superficially, these phenomena are like those due to *Bacterium tumefaciens* which have been studied so intensively by Smith (1911 *et seq.*) and others,—the so-called "crown gall." In reality, they are due to quite a different cause, since bacteria, though sometimes present in

young calluses, are absent in the older ones. The author's microscopical studies show that large quantities of starch (and possibly other food products), produced by the scions, accumulate just above the callus because they cannot pass downward to the tissues of the stock. This huge accumulation of food is the specific cause of the proliferations. Highly differentiated cells apparently produce tumoral formations, while undifferentiated cells apparently give rise to roots or shoots.

The non-utilization of various products of one component of a graft union by the other component appears to be a rather general phenomenon. Daniel (1891) and Vöchting (1894) reported a similar behavior of inulin in grafts among the Compositae. Vöchting grafted *Helianthus tuberosus* (a plant producing inulin) on *Helianthus annuus* (a plant which does not produce inulin) and studied the content of inulin and of starch in the scion and the stock after the union. He found inulin only in the scion and not in the stock. The stock contained only starch.

Moreover, Lindemuth (1878) and Vöchting (1892) have made similar observations on interspecific grafts in the genera *Dahlia* and *Coleus* and in certain varieties of *Beta vulgaris*; i.e., where it was found that the pigmented component cannot affect the color of the non-pigmented one. Winkler (1912) has made comparable observations. All this accords with the present allegation that pigments do not pass the callus.

The cause of the accumulation of scion products above the callus is probably the chemical specificity of the organism rather than the lack of a mechanism by which the products can be transferred to the stock. The author has already shown (Kostoff, 1928) that an antagonism often exists between scion and stock, which is expressed in the acquirement of precipitins in the graft components. This antagonism, which generally varies directly with taxonomic discreteness, is also accompanied by certain morphological phenomena, such as agglutination of the plastids (fig. 3) and necrosis of the stock (fig. 4), in addition to the accumulation of starch above the callus (fig. 23). Since such reactions are observed more in intergeneric than in interspecific graft unions, it seems logical to assume that they occur when the chemical nature of the two plants is quite different. Probably some of the products of the scion are changed into acceptable forms and are utilized by the stock; but other products are not acceptable and therefore tend to accumulate above the callus. This is apparently the reason why no successful union was obtained in attempts to graft *Digitalis purpurea* and *Digitalis lutea* (Scrophulariaceae) with *Nicotiana Tabacum*, *Nicotiana rustica*, *Solanum Lycopersicum*, and *Datura ferox* (Solanaceae); although it is not improbable that interfamilial graft unions may occasionally be made.

It should also be noted that the cambium and its derivative tissues interrupt the callus line first in the lowest zone of a whip-graft union, and it is precisely in this region that there is the greatest accumulation of specific products of the scion. This mode of growth is what is responsible for the

asymmetry of the callus, and therefore, in a sense, for the necrosis, which, in such a union as *Solanum nigrum* on *Nicotiana Sanderae*, appears first in this locus. Even the disappearance of the cells injured during the grafting operation may be a function of these mutual reactions, for acquired lysins were observed *in vitro* in the extracts of *Solanum tuberosum* grafted on *Nicotiana Sanderae*. Thus, all of the evidence tends to indicate the specificity of plants in graft unions.

The accumulation of reserve materials in plant tissues, where certain stimuli are active, is a rather general phenomenon. Thus Küster (1903) has reported the "... enrichment of many cells with albumen and starch under the influence of parasitic fungi or animals." Roncali (1904-1905) has noted the enrichment of insect galls with starch (*Cynips Mayri* on *Quercus* and *Pemphigus cornicularius* on *Pistacia*), and I have observed a great abundance of starch accumulated in the tumors of *Nicotiana* species hybrids (*Nicotiana glauca* \times *Nicotiana Langsdorffii*, *Nicotiana paniculata* \times *Nicotiana Langsdorffii*, F_1 (*Nicotiana rustica* \times *Nicotiana paniculata*) \times *Nicotiana Langsdorffii*, *Nicotiana Tabacum* \times F_1 (*Nicotiana Langsdorffii* \times *Nicotiana Sanderae*), etc.) A similar mutually stimulative activity may be present in the callus also, though it is evidently not so great as in the cases mentioned above. But if the accumulation of starch above the callus plane were merely due to a mutual stimulation of the components, the stock tissue should accumulate starch also, which is not the case. The storage of starch was observed only in the scion above the callus, and the proliferations were manifested only in the accumulation zone.

These statements, however, should not be taken to mean that there is a "specific influence" between scion and stock in the somewhat Lamarckian sense that the phrase is used by Daniel and other earlier writers on the phenomena found in grafted plants. No such "specific influence" between scion and stock has been observed. If formative organizers (Spemann, 1927) or substrata (Goldschmidt, 1927) may pass unchanged through the callus in one or to the other direction, one might expect them to take part in the determination and differentiation of organs on the opposite side of the callus. Such was not the case, however. In about 50 combinations of interspecific and intergeneric graftings in the Solanaceae which the author has carried out at the Bussey Institution, and in about 20 combinations of interspecific and intergeneric graftings of Compositae, Papilionaceae, Cucurbitaceae and Rosaceae which he has carried out in Bulgaria, he has observed no mutual "specific influence" in the determination and differentiation of characters in the scion or stock, beyond the acquirement of precipitins. Possibly the precipitins or other agents of similar nature are responsible for the prevention of "specific influences" between scion and stock by changing the nature of the product of the foreign tissue.

Daniel (1927a, b) has recently published his observations on the progenies from the selfed scions of graft unions in various Compositae. He found

great variation among these progenies, especially among the progenies of *Helianthus tuberosus* grafted on other species of the genus. He ascribes this variability to "specific influence," in the Lamarckian sense, of the stock on the scion. He does not mention, however, any determination of the homogeneity of his *Helianthus tuberosus*. Variability in the progenies of selfed scions in certain graft combinations is sometimes due to non-disjunction in the pollen mother-cell division (possibly in the embryo-sac mother cells also) and to other abnormalities during the maturation division. Such phenomena I have observed in the scions when *Nicotiana Langsdorffii* is grafted on *Solanum nigrum* and when *Nicotiana Tabacum* is grafted on *Datura Wrightii*, but not in the plants from which the scions were cut. Among the progenies of *Nicotiana Tabacum* scions selfed, I have found aberrant types as to their chromosome number. These aberrants do not represent "specific influences" in the strictly Lamarckian sense. But their appearance shows that two plants, proved to be near-homozygous by progeny tests, may give variable progeny after being grafted together, due to abnormal chromosome divisions caused indirectly by the growth disturbance of the grafting process.

SUMMARY

In studying the callus of intergeneric and interspecific simple whip grafting macroscopically and microscopically, the following phenomena were observed:

1. The callus tissues joining the scion and the stock are chiefly the product of the stock.
2. There are large accumulations of starch at the base of the scion just above the callus line.
3. During the growing together of stock and scion, the latter produces various types of proliferations—tumoural formations and numerous small leafy shoots or roots—near the callus line.
4. The callus is asymmetrical, due to the great accumulation of food in the lowest part of the scion.
5. The cambium and its derivatives interrupt the callus line first in the lowest zone.
6. When the scion activity predominates and kills the stock, necrosis begins at the lowest zone of the joining tissues and then spreads in the stock in all directions.
7. The gradual disappearance of the cells injured during the graft operation throughout the union of the graft components is apparently due to certain lytic phenomena *in vivo*.
8. Nests of bacteria were observed in the callus between the scion and the stock plane only in young (15-days-old) calluses.
9. Abnormal vascular cells, either single or in groups, were frequently found in the joining tissues both in the pith region and in the proliferation tissues.

10. Abundant accumulation of sand and groups of crystals of calcium oxalate around the callus were observed.

11. When the tissue of one component is pigmented, the pigment is limited by the callus, and no effect in the other component was observed.

12. The proliferations in the callus, its asymmetry, and other phenomena observed, are interpreted as due to specificity and interactivity of the graft components.

13. While no strictly Lamarckian influence of stock on scion was observed, it was found that certain plants, which before grafting exhibited normal meiosis and produced uniform progeny, after being used as scions exhibited non-disjunction and other abnormal types of meiosis and produced a variable progeny.

The present work was done in the laboratory of Professor Edward M. East while the author was the holder of a Fellowship of the International Education Board. The author is greatly indebted to Professor East for valuable suggestions, advice, and criticisms. He also wishes to thank Professor I. W. Bailey for various helpful suggestions.

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DESCRIPTION OF PLATES

PLATE XXXVII

- FIG. 1. Callus of the union *Nicotiana rustica* grafted on *Datura ferox* (C) with tumoral formation produced by the scion.
 FIG. 2. Callus (C) *Nicotiana Sanderae* grafted on *Datura ferox* with numerous leafy shoots produced by the scion.
 FIG. 3. Callus (C) of *Nicotiana glauca* grafted on *Capsicum pyramidale*; (a) zone with agglutinated plastids.
 FIG. 4. *Solanum nigrum* grafted on *Nicotiana Sanderae* callus (C) with numerous roots produced by the scion.

PLATE XXXVIII

- FIG. 5. Callus (C) of *Solanum Lycopersicum* grafted on *Datura Wrightii*; numerous small tumors appeared on the scion upon the callus, the number and size steadily diminishing toward the upper part.
 FIG. 6. *Solanum Lycopersicum* grafted on *Datura Wrightii*. The callus is described under figure 5.
 FIGS. 7, 8, and 9. Callus of *Nicotiana Langsdorffii* grafted on *Solanum nigrum*. The scions produced tumoral formations around the callus.

PLATE XXXIX

- FIG. 10. Longitudinal section through 4-months-old callus (C) of *Solanum nigrum* (n) grafted on *Nicotiana rustica* (r). Joining tissue (j) produced by the stock.
 FIG. 11. Longitudinal section through the pith of 15-days-old callus (C) of the union *Nicotiana Tabacum* var. *macrophylla* (M) grafted on *Solanum Lycopersicum* (L); (j) joining tissue.
 FIG. 12. Longitudinal section through the cambium zone of 15-days-old callus (C) of the union *Nicotiana Tabacum* var. *macrophylla* (M) grafted on *Solanum Lycopersicum* (L). The photomicrograph is taken of the highest callus region of a simple whip graft.
 FIG. 13. Longitudinal section through the cambium zone of 15-days-old callus (C) of the union *Nicotiana Tabacum* var. *macrophylla* (M) grafted on *Solanum Lycopersicum* (L). The photomicrograph is taken of the lowest callus region of a simple whip graft.
 FIG. 14. Longitudinal section through a 25-days-old callus (C) of the graft union *Solanum Lycopersicum* (L) grafted on *Nicotiana glauca* (g). The callus is interrupted by the cambium and its products; (v) group of abnormal vascular bundles formed in the joining tissue.
 FIG. 15. Group of abnormal vascular bundles from the pith joining tissue of 50-days-old callus of the graft union *Nicotiana rustica* grafted on *N. glauca*.
 FIG. 16. Longitudinal section through a 50-days-old callus (C) of the graft union *Nicotiana rustica* grafted on *N. glauca*. The callus (C) is interrupted by the newly produced vascular cells formed in the joining tissue.

PLATE XL

- FIG. 17. Section through the tumoral formation of the scion of a 4-months-old callus of the graft union *Solanum nigrum* grafted on *Nicotiana rustica*.

FIG. 18. Longitudinal section through 4-months-old callus (C) of the graft union *Solanum nigrum* grafted on *Nicotiana rustica*. (v) vascular bundles; (C) callus interrupted by the vascular bundles; (j) joining tissue.

FIG. 19. Longitudinal section through the pith of a 50-days-old callus (C) of the graft union *Nicotiana rustica* grafted on *N. glauca*; (j) joining tissue.

FIG. 20. Longitudinal section through the pith of a 5-months-old callus (C) of the graft union F_1 (*Nicotiana Tabacum* x *Nicotiana Rusbyi* (F) grafted on *N. Sanderac* (S); (i) joining tissue.

PLATE XLI

FIGS. 21 and 22. Longitudinal sections through the woody region of a 5-months-old callus (C) of the graft union *Nicotiana Rusbyi* grafted on *N. rustica*; (j) joining tissue.

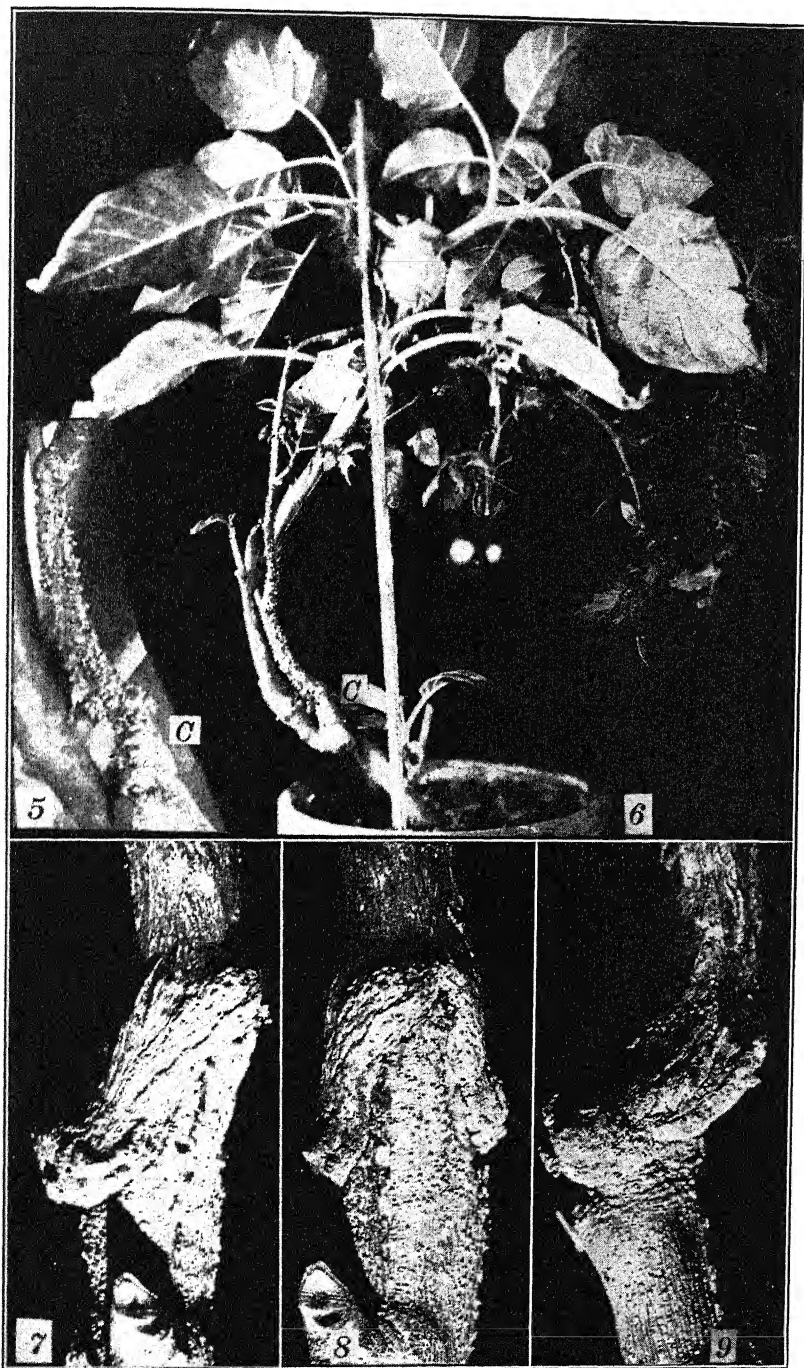
FIG. 23. Longitudinal section through a 25-days-old callus of the graft union *Solanum Lycopersicum* grafted on *Nicotiana glauca*. The scion contains abundant accumulation of starch.

FIG. 24. Photomicrograph in polarized light from tissue near to the callus line of a 4-months-old callus of the graft union *Solanum nigrum* grafted on *Nicotiana rustica*. The active substance in the polarized light accumulated in the single cells is sand and groups of crystals of calcium oxalate.

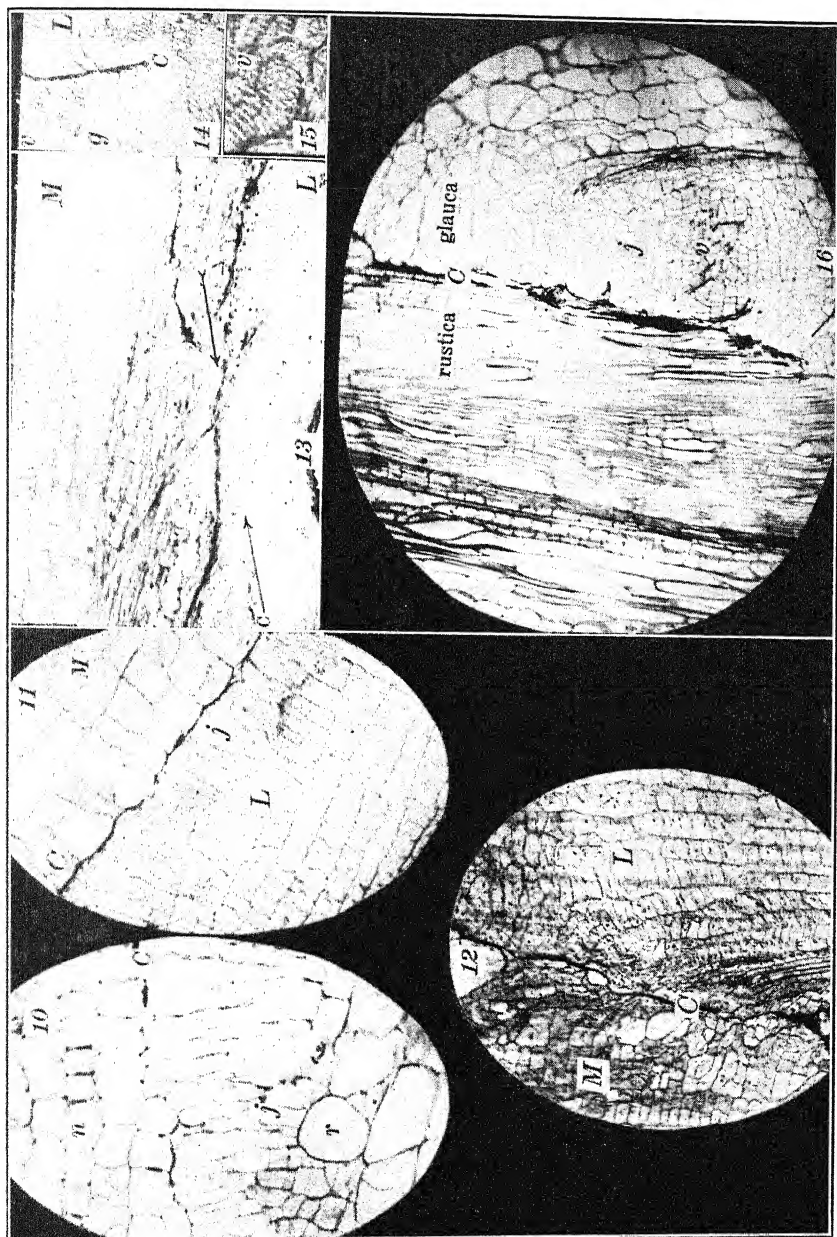
FIG. 25. Longitudinal section through a 15-days-old callus (C) of the graft union *Nicotiana Tabacum* var. *macrophylla* grafted on *Solanum Lycopersicum*; (b) bacterial nest.



KOSTOFF: CALLUS TISSUE

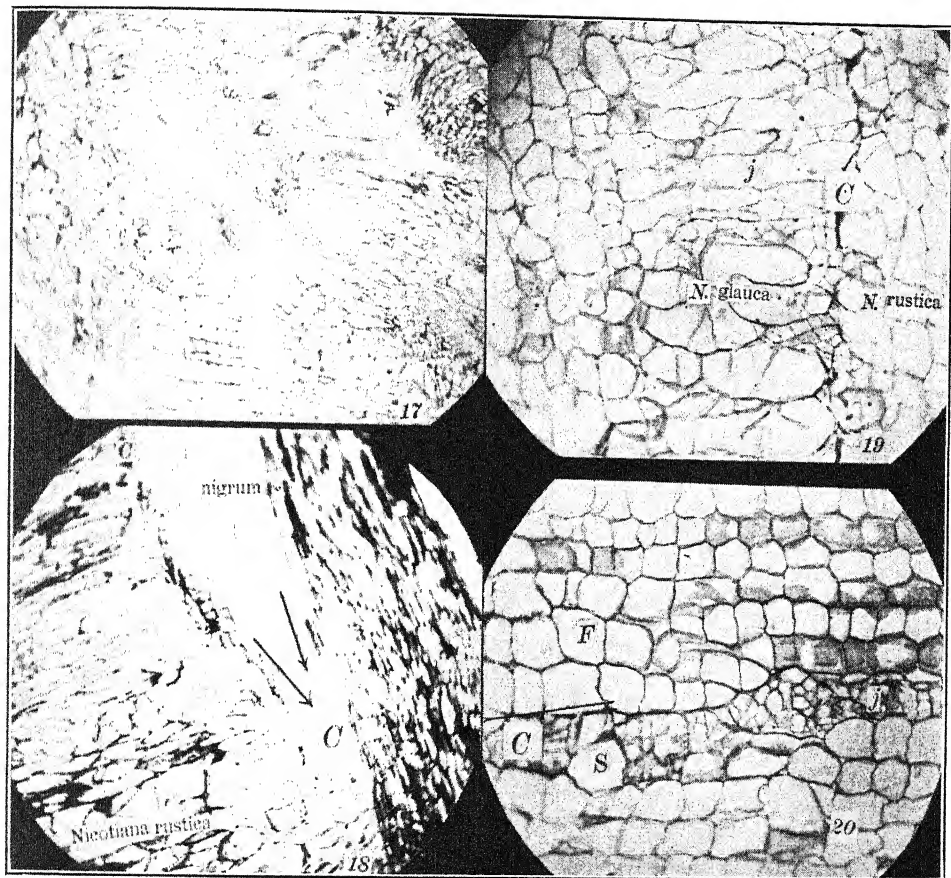


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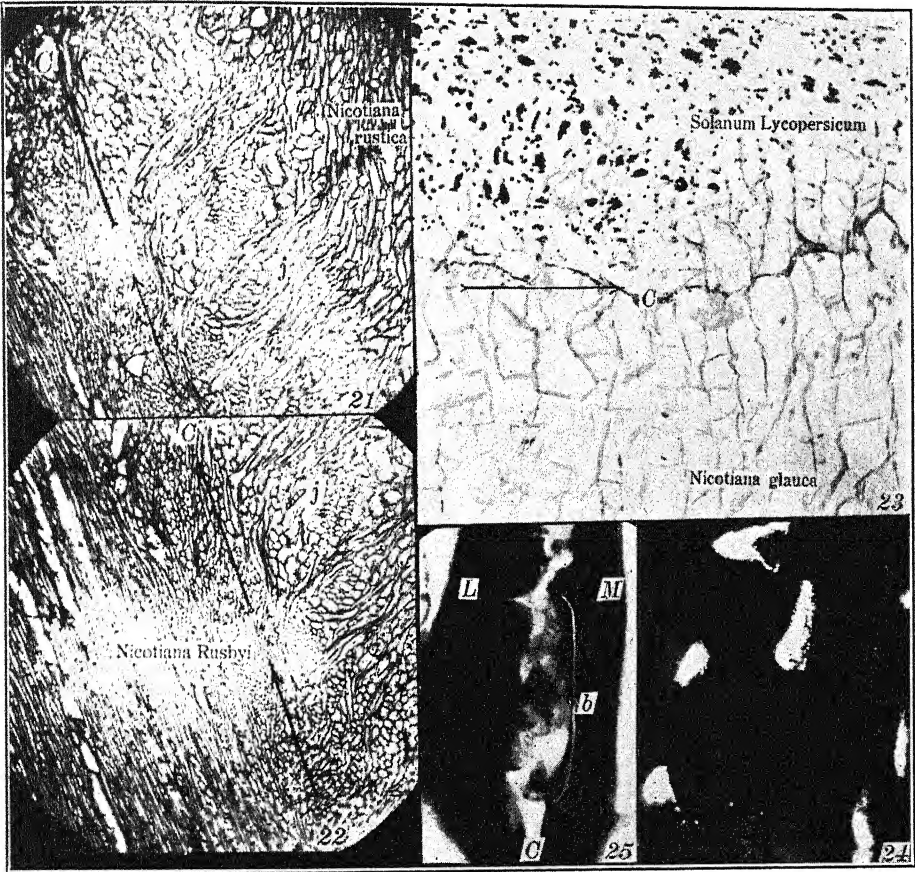


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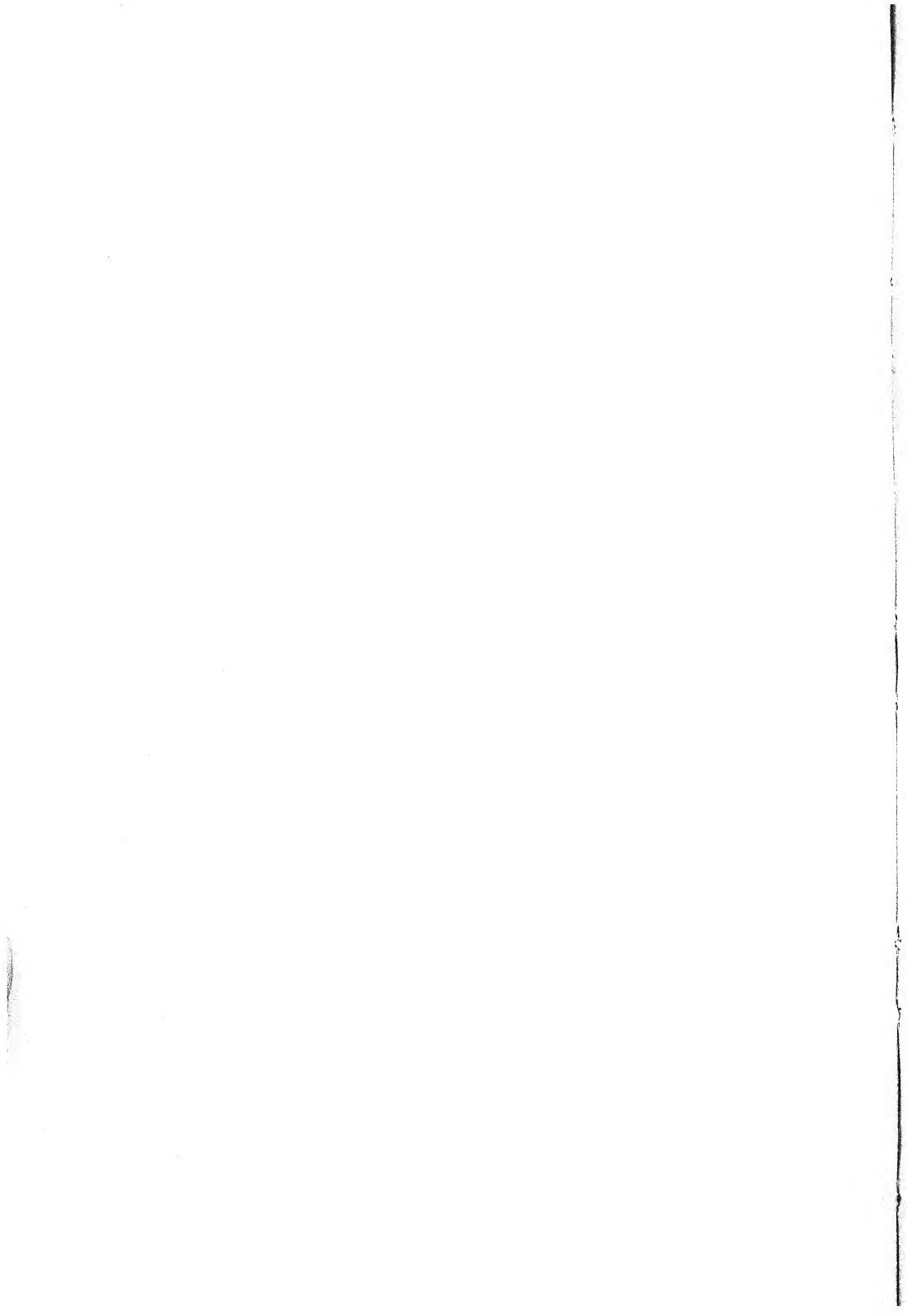




KOSTOFF: CALLUS TISSUE



KOSTOFF: CALLUS TISSUE



A SPECIES OF *ACROTHRIX* ON THE MASSACHUSETTS COAST

WM. RANDOLPH TAYLOR

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A quotation from the old but yet invaluable work by Farlow (3, p. 8) on the Marine Algae of New England will indicate the striking character of the discovery to be reported in the present note: ". . . Hereafter any advance in the knowledge of our marine algae must be made by a careful microscopic study on the shore. Probably all the large and striking species are now known, or if any remain to be discovered their discovery will be by mere chance, and not by any systematic search." More than 50 years have passed since this opinion was expressed, and it can hardly be said to have been without justification. A number of fairly large algae have been reported of recent years, but almost exclusively within genera that were inadequately understood in Farlow's time, such as *Cladophora* and the crustaceous coralline algae, which while not of microscopic proportions yet require a microscopical study of structure or measurements for correct classification. But in each of two seasons past there has appeared at Wood's Hole a striking alga: in 1927 a species of *Asparagopsis* representing a family of Rhodophyceae not known to this coast, and in 1926 and 1927 a species of *Acrothrix* representing a genus of Phaeophyceae heretofore unknown from America.

The earliest collection of this *Acrothrix* was probably taken from Penikese Island, in the outer part of Buzzard's Bay, Massachusetts, and consisted of 2-3 rather battered specimens, collected in 1925, but not fit for a careful study. It was secured there again in 1926, rather more abundantly and in better condition, and in July 1927. However, by far the best material was obtained drifting along the shores of Nobska Point, north-east of Wood's Hole, between July 5-10, 1927. The writer has examined microscopically about 100 plants, finding almost all in fruit, and these without exception with unilocular sporangia. The original material appeared among the collections of students in the class in algae, and it was recognized as of peculiar interest by Dr. I. F. Lewis, who generously granted the present writer the privilege of studying it.

In habit the Massachusetts plants agree very well with those from Bohuslän on the Swedish west coast, where they were originally collected. The main axis is usually somewhat flexuose or slightly angled, and the lower branches often tend to equal or over-top it. The plant is rather stiff in the lower portions, and certainly not gelatinous. In these gross features the correspondence with the Swedish plant is complete. On the other hand the

Massachusetts specimens branch more freely and are lighter in color, at least in comparison with the large and typical specimen in the writer's herbarium from Kristineberg in Bohuslän, collected and determined by Kylin, who originally defined the genus and the other known species. In structure the plants agree well with the descriptions and figures of *A. gracilis*, as given by Kylin (4) and by Oltmanns (5, pp. 35-40), showing the type of apical growth that he illustrates in his figure 331. The differences that occur will be described later, and are certainly of no more than specific value, being practically limited to differences in the contour of the assimilative filaments, in the shape and size of the sporangia, and in gross features. However, since the American plant is not in absolute accord with that from Europe it appears better to consider it distinct for the present, pending the appearance of completely intermediate types. The only American species that would by any chance be mistaken for *Acrothrix* are in the genera *Dictyosiphon* and *Chordaria*. The habit of branching will distinguish it from the former, where the branches are comparatively short and the structure is entirely distinct. From the latter it differs in its lighter color, different habit of branching (more secondary and tertiary lateral branches), lack of mucilage, and particularly its structure, since it has a single axial strand. A complete description of the Massachusetts plant is as follows:

Acrothrix novae-angliae n. sp.—Plant loosely tufted, light brown in color, to 25 cm. tall and about 40 cm. in diameter; smooth but not slimy, somewhat flaccid in the distal parts, but comparatively rigid toward the base and collapsing very little on being removed from the water; attached by a rather thin, lobed, disciform hapteron; branching radial, alternate, to 3 (occasionally 5) orders, in places appearing dichotomous, members of all orders frequently somewhat contracted at the base, otherwise cylindrical except that the ultimate members taper to an acute apex, 0.5-0.75-1.50 mm. in diameter, the main axis soon dissolving into long primary branches which may equal or exceed it in length; secondary or tertiary branches inserted at about 60° to the supporting axis, somewhat angularly bent, usually exceeding the long (to 10 cm.) alternate flexuous flagelliform or subfalcate branches of the succeeding order which are naked or bear a few short (1-2 cm.) curved ramuli; at the base of the plant several short, very slender primary branches may be present, especially rigid and rather closely branched; structurally the older parts show a subparenchymatous cortex about the axial cavity, the inner cells large and colorless, the outer or peripheral cells much smaller and with a few small chromatophores; surface loosely clothed with chromatophore-bearing assimilatory filaments, which arise singly or in pairs from some of the outer cortical cells, if single frequently 2- rarely 3-branched, straight or more often arcuate, 3-5-6-9 cells long, the lower or basal cell short, the next frequently elongate, the upper cells moniliform, often asymmetrical, 6.3-8.0 μ in average diameter, and 0.75-1.50 times as long as broad; colorless hairs not abundant, having an average diameter of 7.7 μ , replacing branches of assimilative filaments; sporangia usually attached to the basal cell of the assimilative filaments, generally single, but occasionally up to 3 sporangia and one assimilative filament attached to a single basal cell, at first ovoid, later subspherical and 21.7-26.75-30.7 μ in diameter, unilocular, at maturity producing numerous

zoöspores which are round-ovoid, about 4.6μ in greatest diameter, and show one large chromatophore and a very prominent eyespot. Type Herb. W. R. T., No. 13234, coll. J. J. Copeland drifting off Nobska Pt., Wood's Hole, Massachusetts, July 8, 1927.

In old branches the assimilative filaments are more sparse than on the vigorous young shoots. They are also less moniliform, more slender, and shorter, often consisting of but 3-4 cells, perhaps representing regeneration or replacement of the original ones. Hairs are few, and practically absent from the older parts. The sporangia may be sessile upon the outer cells of the cortex or terminal upon a one-celled stalk. In both old and younger shoots an initial from an outer cortical cell or the basal cell of a branch may ramify for a short distance up and down the axis, giving off assimilatory filaments at right angles, and containing chromatophores itself, although appressed to the axis.

The notable distinguishing feature of *Acrothrix* is the type of development from the growing apex. As originally described, a colorless hair terminates the shoot, which develops from a meristematic cell or cells in the axial row. Behind this point the axial row develops lateral cells from each segment, these forming the assimilatory filaments and cortex while the cells of the axis simply elongate. Oltmanns, however, considers that the axial row is not necessarily tipped by a hair, the alternative condition being one in which the shoot is terminated by the meristematic cell, or with a few short distal cells representing the missing hair. Since the difference between these two conditions is that of trichothallic *versus* apical cell growth the contrast is extreme unless one is willing to accept that under certain conditions the hair becomes deciduous and centrifugal growth slackens and stops. Such indeed appears to be the case, and no difference is visible in the progress of development of the branch behind the growing point. From *Halorhiza* and *Stilophora* an important difference is evident in that only one instead of 4-6 axial cell rows are present.

The writer found it quite impossible to determine the growth method of his material from crushed tips, and was forced to imbed and section the samples. Tips and older portions were fixed in Flemming's solution, imbedded without any difficulty in celloidin, and serial sections prepared by the method of Carothers (1). The serial feature proved essential because the apex is so slender as to be practically impossible to locate in a dish of mixed sections. The absence of mucilage made it easy to secure perfect infiltration of the tissues, as had also been the writer's experience with *Castagnea virescens* preserved in formaldehyd, which shows the typical Chordariacean structure splendidly in thick celloidin sections while it is nearly unintelligible in the thinner paraffin sections.

The tips available were all from mature plants, and to this is perhaps attributable the fact that all studied were of the type lacking the terminal hair. Growth appeared to be effected from the outermost (or apical) cell

itself or at most from the cell next below it. Segments of this then immediately cut off a pericentral series which according to Kylin's description should have formed elongated primary assimilative filaments. Instead of this they immediately enlarged, and characteristic primary assimilators were either absent (Pl. XLIII, fig. 1) or reduced to about 2 cells (fig. 2). These enlarged segments (to about 6-9 in number) formed the primary cortical layer about the original axial row segment, and bore directly the secondary assimilative filaments (fig. 3). Within two or three segments of the apex the cortex begins to loosen from the axial filament, forming a medullary cavity which at first is inconspicuous (figs. 1, 3) and later comes to occupy half of the diameter of the branch (fig. 5). The original cortical series divide to form a second well marked peripheral cortical layer, which in turn may develop an incomplete third layer figs. 4, 5). In either case the outermost bear the basal cells of the assimilatory filaments. The original cortical cells may also divide transversely, but in general seldom divide and become much elongated, to an average of $470\ \mu$ in length and $125\ \mu$ in diameter. The walls of these cortical cells do not become greatly thickened by gelatinization. The axial cells do not seem to divide transversely, and become very long in proportion to their diameter, which becomes rather diminished by stretching (figs. 3-5). As the medullary cavity increases the axial filament becomes separated from most of the cortex, maintaining as a rule an attachment with one side or another so as to wind down the wall in a more or less tortuous course. While it may become somewhat distorted by opposing adhesions and thus occasionally simulate lateral branches, these are short and not comparable to the regular whorls of branches reported in *Spermatochneus*. The cavity is filled with gas rather than mucilage.

The assimilative filaments arise singly or 2-3 from a basal cell upon the periphery of the cortex, and rarely branch but once (figs. 6-9). Colorless hairs are not common in the Massachusetts material, nor very long, and are of about the same diameter as the assimilatory filaments (fig. 10). The sporangia are nearly always found with an assimilative filament upon a common basal cell (figs. 11-14). At first they are narrowly oval, becoming nearly (fig. 14) or quite spherical (fig. 13) at maturity. The zoöspores (fig. 16) are very numerous, and are discharged by the opening of the summit of the sporangium (figs. 11, 14). There are occasionally 2 sporangia (and then of different ages) upon the same basal cell (figs. 11, 14). Occasionally a vegetative outgrowth is regeneratively established from the base of a sporangium (fig. 15), but no general replacement of sporangia by new ones from the base within, as is not infrequent in algae, was noted. Gametangia, presumably plurilocular structures, were not noted in any of the very many specimens tested microscopically.

Plants of *Acrothrix novae-angliae* adhere well to paper, except perhaps in the oldest parts near the hapteron, and darken quite considerably in drying, the color then ranging from a light brown toward an olive green. At first

it was thought that there was no tendency on the part of the alga to discolor the paper on which it was mounted. In 1927 two or three large specimens, when dried, showed no discoloration about them, even after several days storage in the dark. However, they were spread about a desk for study on a damp day in a diffusely lighted room, and within a few hours developed a very considerable purplish-brown discoloration of the paper about the branches, which appeared especially deep when held against the light. Some soluble substance therefore appears to be discharged from the plants during drying which diffuses out upon the wet paper, permeates it, and under suitable illumination and humidity darkens as indicated.

This species was entirely secured from material drifted ashore after periods of rough water. However, something of its habitat is indicated by the fact that several small specimens were attached to old and rather decayed fragments of *Zostera* rhizomes. The hapteron of another was attached to a piece of *Dermatolithon pustulatum*. It appears therefore that the plant is an inhabitant of comparatively shallow water, probably frequenting the extensive *Zostera*-beds common along the coast. If the assignment of the genus to the Stilophoraceae by Svedelius (in Engler and Prantl, 2) is correct it is to be expected that a somewhat similar sexual generation will be found in time, probably at a somewhat different time of year. The same is true if Kylin's original disposition of it (in the Chordariaceae, but which also included *Stilophora*) is more correct. However, it is not beyond the bounds of possibility that the sexual generation differs very much in appearance, so that a closer approximation to the Dictyosiphonaceae in classification would be necessary, in view of the fact that the sexual generation of the Dictyosiphonaceae is less elaborate than the sporophyte. The structure of the sporophyte is against this latter possibility, showing much greater similarities to the Stilophoraceae and Chordariaceae.

The distinguishing features that enable a person to differentiate between *A. novae-angliae* and the earlier *A. gracilis* may be outlined as follows: In diameter of the axis and branches it appears that the American plant is somewhat sturdier than the European one, since Kylin gives a diameter of 0.5 mm. as compared with ours, which averages about 0.75 mm. and may reach 1.5 mm. However, the specimen of *A. gracilis* in the dried condition at the writer's disposal has a maximum diameter of nearly 1.0 mm. so that Kylin seems to have secured specimens subsequent to the original discovery and description that in the emergency of a critical revision might call for a modification of this—and perhaps other—descriptive statements. Since the primary assimilators were ill developed in the Massachusetts material a comparison with that feature of *A. gracilis* is impossible, beyond this statement. The secondary assimilative filaments in *A. gracilis* have a length-range of 4-7 cells, while those of *A. novae-angliae* range more widely (3-9 cells), this being probably of little importance. In diameter there is also little difference, but Kylin attaches more importance to the asymmetric-

ally swollen character of the outer assimilatory cells in *A. gracilis* than the writer is justified in doing in the case of *A. novae-angliae*, where the asymmetry is very moderate in comparison with that figured by Kylin (his fig. 23). The sporangial diameter for *A. gracilis* is given as 18–22 μ , while *A. novae-angliae* ranges between 21.7–30.7 μ , averaging 26.7 μ and so is greater than that of the European plant. However, the length of the sporangia reverses this state, since the Massachusetts plant's sporangia are nearly spherical and Kylin cites a range of 28–33 μ for the length of sporangia in his plants. The sporangia in the Massachusetts material appeared more oval in the contracted imbedded material than in the crushed living specimens, in which the discharge of the zoöspores was watched. The most pronounced difference between the European and the American material seems to be the much greater extension of the branch-system in the latter, for Kylin assigns lateral branches to but the second order to *A. gracilis* and no more appears in the specimen at hand. *A. novae-angliae* consistently shows much more abundant development of branches of the second order, which are quite infrequent in Kylin's plant. The branching is in fact abundant to the third order, occasional to the fourth, and little branchlets of the fifth order were seen. This produces a plant of much bushier habit. The primary branches tend to exceed the main axis in both species, but later ramifications do not tend to equal in length the axes which bear them. Branches of all orders are somewhat contracted at the base in both species. The single available specimen of *A. gracilis* is distinctly darker than any of the *A. novae-angliae* material.

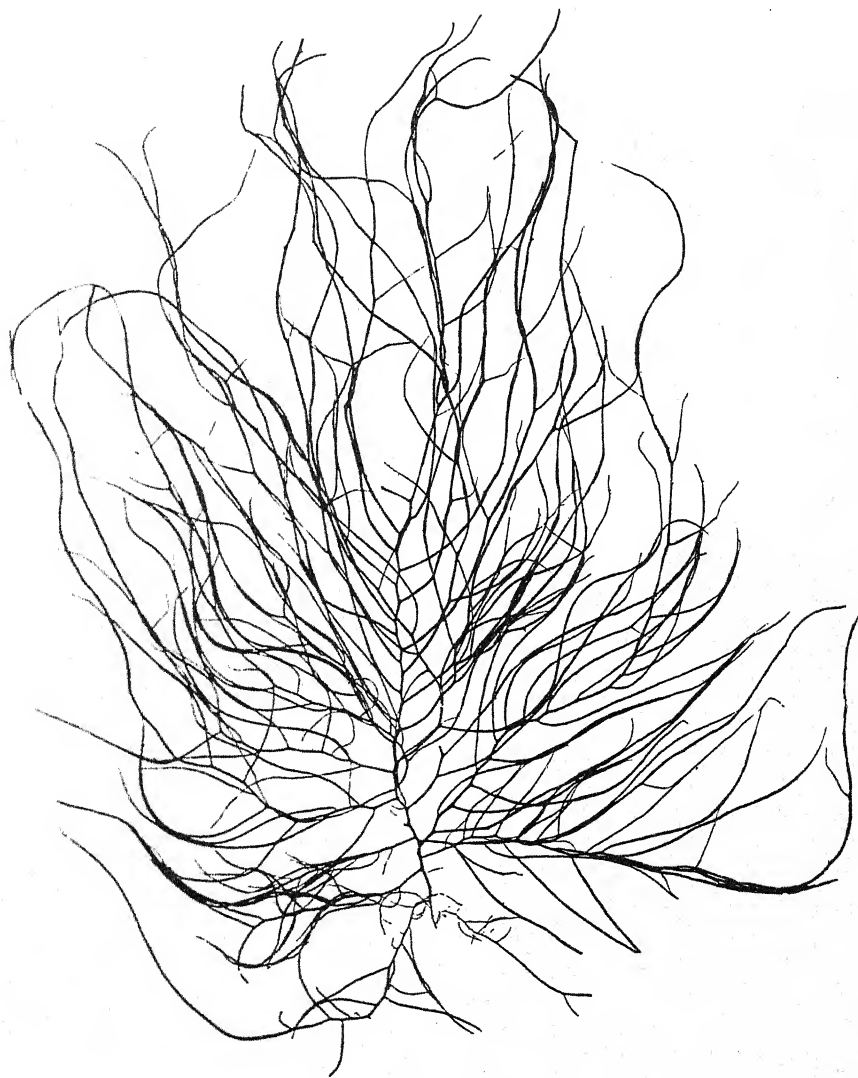
SUMMARY

Acrothrix novae-angliae is described from material collected on the coast of southern Massachusetts. This plant develops morphogenically from an apical cell or cell row, producing an axial filament surrounded by an apparently parenchymatous cortex that ultimately enlarges to form a medullary cavity down the wall of which the axial filament extends. The cortex is loosely covered with assimilatory filaments, the basal cells of which may also bear unilocular sporangia. This species differs from the previously described *A. gracilis* Kylin (type of the genus) in being more extensively branched and having subspherical rather than oval sporangia.

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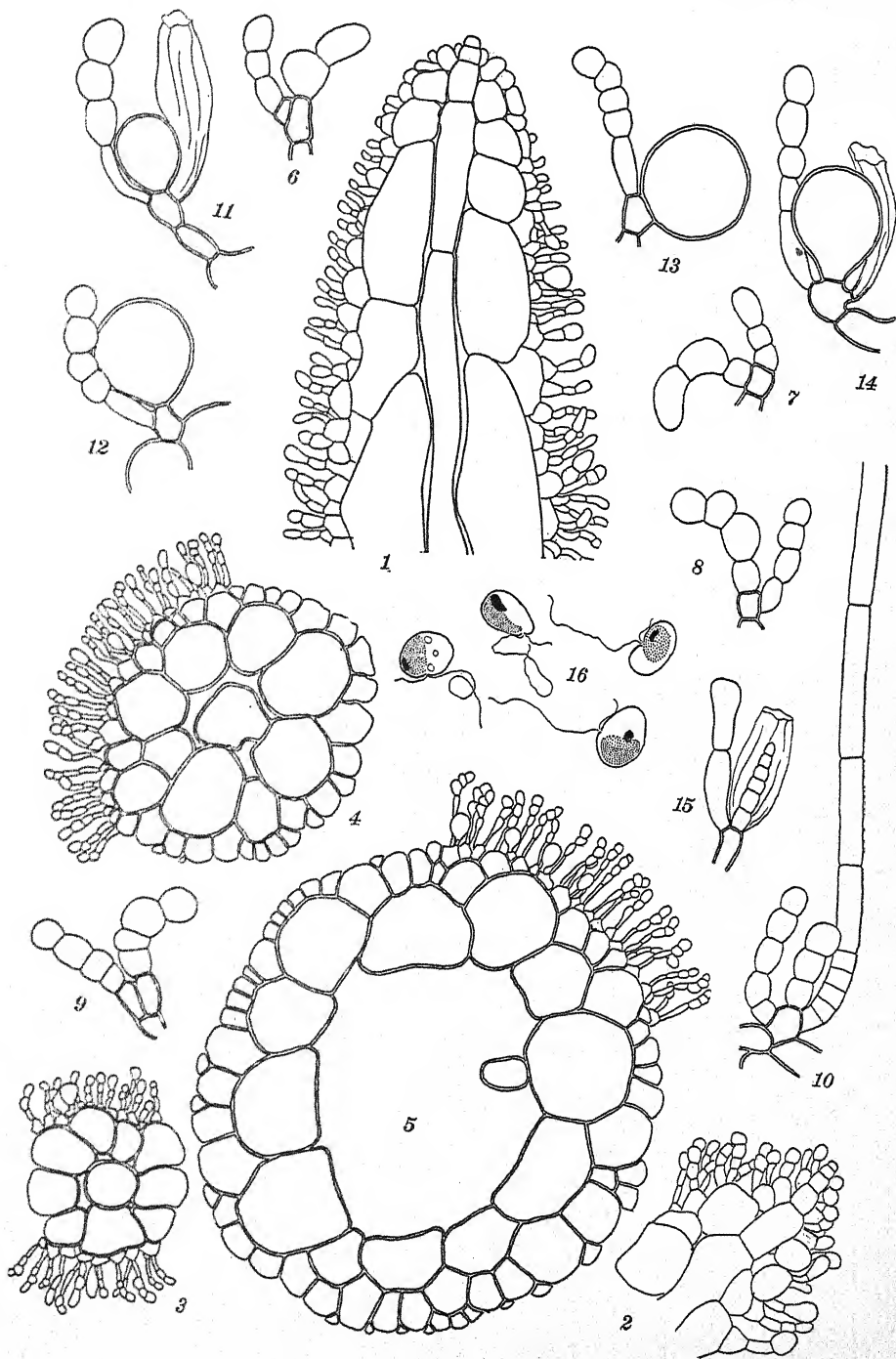
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TAYLOR: ACROTHRIX





TAYLOR: ACROTHRIX



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DESCRIPTION OF PLATES

PLATE XLII

Habit of plant of moderate size showing branching to the third order. No. 13465, Penikese Island, Buzzard's Bay, Mass., coll. J. F. Grant, July, 1926. This plant was selected for reproduction rather than the type because, being less extensive, it required a more moderate reduction to conform to the size of the plate. $\times 0.62$.

PLATE XLIII

FIG. 1. Apex of branch, longitudinal median section, showing method of development of cortex and assimilative filaments. $\times 310$.

FIG. 2. Apex of branch, longitudinal median section, showing less immediate consolidation of the cortical elements and more evidence of distinctive primary assimilative filaments. $\times 355$.

FIG. 3. Transverse section of branch, showing the early separation of the cortical from the axial cells, and but one cortical layer clearly marked. $\times 190$.

FIG. 4. Transverse section of branch, showing more advanced separation of the cortical cells and the development of a second cortical layer. $\times 190$.

FIG. 5. Transverse section of a fairly mature branch, showing a well developed medullary cavity with the axial filament lying on the right-hand side against the cortical wall. A partial third cortical layer is now present. $\times 190$.

FIGS. 6-9. Assimilative filaments, types showing a single branching, in various relations to the basal cell. $\times 575$.

FIG. 10. Secondary colorless hair with assimilative filaments on a common basal cell. $\times 575$.

FIGS. 11-14. Sporangia and assimilative filaments in various relations. $\times 575$.

FIG. 15. Formation of a filament of cells from the base of an empty sporangium. $\times 575$.

FIG. 16. Group of four zoöspores. $\times 1150$.

The zone of assimilative filaments in figures 3-5 is drawn over only part of the circumference, and is slightly diagrammatic, as it is also in figure 1.

GENERA OF NORTH AMERICAN FABACEAE

V. *ASTRAGALUS* AND RELATED GENERA

P. A. RYDBERG

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Linnaeus in the first edition of the *Species Plantarum* admitted two genera, *Phaca* and *Astragalus*, the former containing 3 species, the latter 33. Turning to the fifth edition of his *Genera Plantarum*, which was published a year later and which better illustrates Linnaeus' concepts of genera than does the *Species Plantarum*, we find that the most essential character which separates *Phaca* from *Astragalus*, is the 1-celled legume of the former and the 2-celled one of the latter. The partition dividing the pod of *Astragalus* is formed by the invagination of the edges of the valves at the lower suture. In certain species, regarded by some as belonging to *Phaca*, the lower suture is infolded, forming a deep sulcus, but in *Astragalus* the development has gone a step farther so that the backs of the two valves have fused along the lower edge of the pod, thus forming a double-walled septum. In those species, which the writer regards as representing the genus *Astragalus* proper, this septum meets the upper suture of the pod and fuses with the same. This is the case in most of the species included in *Astragalus* by Linnaeus. In a few of them the septum does not extend to the upper suture and forms only a partial partition, as for instance in *A. alpinus*. Such species have been referred by some of the later authors to *Phaca*. In others the partition is formed by the intrusion of the upper suture instead of the lower. These constitute a part of *Oxytropis* DC. In some species of *Cystium*, the partition is formed in small part by the upper suture also. In the genus *Astragalus*, as treated by Bunge, Boissier, Benthams and Hooker, Gray, and later by Taubert in the *Pflanzenfamilien*, where even *Phaca* L. and *Tragacantha* Tourn. are merged in it, the pod has been allowed such an unlimited variation that even *Homalobus* Nutt. and *Kentrophyta* Nutt. have been included. These two genera do not even agree with the characters given by Benthams and Hooker or by Taubert as distinguishing the subtribe *ASTRAGALANAE*. Using their keys or synopsis, anyone would place these genera in the *ROBINIANAE*.

The first segregation from *Astragalus* was done by Necker in 1780, in his *Elementa*, where *Aragallus* and *Spiesia* were published. The former was segregated from *Astragalus* by the subcaulescent habit, and based on 12 species constituting one group of that genus in the 14th edition of Linnaeus' *Systema* (edited by Murray). There is not any doubt as to what constituted Necker's concept of the genus, but it is impossible to determine which

species should be regarded as the type. Greene contended that the genus is the same as the later *Oxytropis* DC. and should supplant it. This is not true, however, for only 4 out of the 12 species belong to De Candolle's genus. Further notes will be given under *Oxytropis*. *Spiesia* Necker was based on some species of *Phaca* of Pallas but which one is not stated. Kuntze and Greene contend that it was *P. muricata* and hence also a part of *Oxytropis*.

The next botanist who expressed in print his dissatisfaction with the limits of *Astragalus* was Medicus. He gave lectures before the Physico-economic Society of Kurpfaltz, which were printed (Vorles. Kurpf. Phys.-Oek. Ges. 2: 374-378) in 1787. Besides admitting *Phaca* L. and *Tragacantha* Tourn. (restored by Miller), he established *Glandula*, *Glottis*, *Hamosa*, *Onix*, *Stella*, *Tium*, *Triquetra*, *Contortuplicata*, and *Glaux*. It is unfortunate that his work has mostly been disregarded by later botanists. This may be due to its scarcity. I do not know whether a copy is to be found in America, but at least I have not seen the work. My information concerning his concepts is gathered from a later publication, his Philosophische Botanik of 1789. In this work the discussion is rather fragmentary; and to one that has not given special study to the structure and great variation of the pod in this group, it is hardly convincing. Most of his genera fall in the group having a perfectly 2-celled pod. In *Astragalus* proper the pod is short, turgid, and leathery or woody in texture. In his Philosophische Botanik, Medicus does not admit the name *Astragalus* and it is not possible to tell which one of the genera he regards as the typical *Astragalus* of Linnaeus. As *Glandula*, *Glottis*, and *Stella* have essentially pods of the same structure, I regard them as synonyms of *Astragalus*. The other genera (except *Triquetra*) I regard as well-founded segregates. In *Hamosa* the pod is 2-celled, leathery, cylindric or often flattened, but much elongated. In *Glaux* the pod is papery in structure and much inflated. Unfortunately the name is preoccupied by *Glaux* L. Instead of *Glaux* Med. we must use a later name, *Cystium* Steven. In *Onix* the pod is also inflated, of somewhat firmer texture, but stipitate. In *Contortuplicata* the pod is elongate as in *Hamosa*, but of a more delicate texture and splits through the septum into two strongly falcate, flat, false carpels, the valves of each made up of one of the valves and its part of the septum. *Tium* is the only segregate which has a partial septum. It has an elongate pod as *Hamosa* but is distinctly stipitate in the original species. Medicus placed it among the genera with 1-celled pods. It was based on *Astragalus sulcatus*. There is another segregate with partial septum found in Europe represented by *A. alpinus*. This was not treated by Medicus. Evidently he regarded the species as having a 1-celled pod and as belonging to *Phaca*.

In 1802 De Candolle published his Astragalologia, in which *Oxytropis* was proposed (See pages 3, 19, and 53). This was based on species of *Astragalus* having a beaked keel and a pod more or less 2-celled by inflexion of the upper suture. This genus has generally been accepted.

In 1832 Rafinesque proposed the genus *Physondra* (Alt. Jour. 145), based on *Psoralea longifolia* Pursh., which is a true *Phaca*, and *Orobis dispar* Nutt., which is a *Homalobus* and does not fit Rafinesque's diagnosis. The former must therefore be regarded as the type, and *Physondra* becomes a synonym of *Phaca*.

In 1832 Christian Steven published a paper in the Bulletin de Soci  t   Naturelle of Moscow (vol. 4) in which he divided *Astragalus* into 24 tentative divisions. These have usually been regarded as genera, because he gave them noun names. They should rather be regarded as sub-genera, as he listed all the species as species of *Astragalus*. In 1856, he took up the work again and in the same publication (vol. 29). He distinctly defined some of these sections as genera, and added a few more. The publications of these genera must therefore be counted from 1856. The Russian-Siberian species were then divided into 17 genera, of which 13 were new. Of these I need to consider only the following: *Hedyphylla*, based on *Astragalus glyciophyllus* and *A. glyciophylloides*, the former of which is introduced in America; *Cystium*, a name which should be substituted for *Glaux* Medicus, based on the same species and untenable; and *Xerophysa*, which cannot be separated generically from the *Cystium*. The rest are made up of groups of species which are not represented in America or which should be regarded as belonging to *Astragalus* proper. Steven's work has not been adopted by botanists.

In 1838 Nuttall published in Torrey and Gray's Flora of North America the genera *Homalobus* and *Kentrophyta*. As stated before, these two genera do not agree even with the characters of the subtribe ASTRAGALANAE, as the pod is laterally compressed and not at all 2-celled. There was also published a subgenus *Orophaca* of *Phaca* with the remark "(an gen.?),\" indicating that the author wondered if it should not be regarded as a genus. This was accepted by Britton as a genus in the Illustrated Flora.

In 1868 and 1869 appeared, in the Memoires of the St. Petersburg Academy, A. Bunge's important monograph of the genus *Astragalus*, exceedingly valuable in many respects especially as the author had tried to include all known Old World species, and as it contains elaborate keys, usually ample descriptions, valuable notes, synonymy, distribution, and collectors' names. These many good qualities and the fact that it has been the only one comprehensive monograph of the genus in the Old World, has had the result that this revision has been followed more or less up to this day; but as to the real understanding of the genus it has caused considerable harm. Bunge's classification, followed by Boissier, and lately by Taubert, is in my opinion untenable. The shortcomings of this work were perhaps caused by the fact that the mature fruit of perhaps half of the species was not known to the author and his classification was therefore naturally very artificial. He also used characters which were of no taxonomic value in showing relationship, as, for instance, the pubescence. He divided the

genus into 8 subgenera. First he set off *Pogonophace*, containing many Asiatic and two Abyssinian species, by the hairy stigma, which character alone would suggest the subtribe COLUTANEAE. This subgenus contains probably more than one distinct genus. The 7th section of it, *Caraganella*, for instance, contains one species which has abruptly pinnate leaves as *Caragana* but a long-stipitate pod. Next, Bunge set off the annual species as a subgenus *Trimeniaceus*, based on a character of no sectional taxonomic value. In this subgenus he included species with either simple or malpighiaceous hairs (2-branched hairs, apparently fixed at the middle). This very character he used in dividing the 6 remaining sections into two groups. The character might be, under certain circumstances, of some value readily to distinguish natural groups based on some other character, but it is useless in *Astragalus* for it would place closely related species in separate sections, as for instance here in America *A. stiatius* and *A. goniatus*, and both kinds of hairs might be found in the same species. Bunge also segregated the subgenus *Tragacantha* on account of the turbinate sessile calyx and spinose-tipped abruptly pinnate leaves. This I regard as a good genus, as did Tournefort, Miller, and others. Another extremely artificial and worthless character is used in distinguishing the sub-genus *Phaca*, with the flowers in lax racemes, or axillary or "subradical" flowers, from *Hypoglottis*, with the flowers in dense heads. In other words, Bunge in his key to the sub-genera mixed characters, some of which may be regarded as even generic, with others scarcely specific. As a tentative arrangement, Bunge's classification might have been useful just as Linnaeus' sexual system served its time, but it is astonishing that Taubert in the Pflanzenfamilien could not find any better one for the Old World species. For the North American species he followed Gray's arrangement, which in general was based on true relationship. It was unfortunate that Gray was so influenced by Bunge as to merge *Homalobus*, *Kentrophyta*, and *Phaca*, all admitted in Torrey and Gray's Flora of North America. He should instead have added a few more. Another criticism I may make of Bunge's work is that he did not pay enough attention to previous authors (except Pallas and De Candolle). He does not even cite the only three original species of *Phaca*, which Linnaeus had in his first edition of the Species Plantarum, viz. *P. boetica* L., *P. alpina* L., and *P. sibirica* L. He gives as a synonym of two species *P. alpina*, not in the sense of Linnaeus but as used erroneously by two other authors.

The other segregates made during the nineteenth century by Walpers, Regel and Schmalhausen, Hochstetter, Opiz, and Royle, need not be considered, as they were made on Old World groups or single species not represented in North America. Several of these may deserve generic rank. Fourrier in the Annals of the Linnean Society of Lyons II, 16, in 1868, proposed several new names for older genera. The only genus of interest to us published on Old World species is *Erophaca* Boiss. (Voy. Bot. Espagne 176, 1839-45), based on *Phaca boetica* L., the first species published in the

Species Plantarum. As some have regarded this as the type of *Phaca* and as it probably is not congeneric with the other two species, it might cause trouble in nomenclature. It is better, therefore, to accept *P. alpina* as the type, as that represents the concept of such botanists as Pallas, De Candolle, Hooker, and Bunge. The latter makes the subgenus *Phaca* from *P. frigida* L. (which is the same) and its relatives.

The bilocular condition of the pod in *Astragalus* proper has arisen in the following manner. The lower suture of the ovary (corresponding to the midrib of the foliar leaf) has not only become inflexed, as we find it in some species of *Phaca*, where the lower suture is strongly sulcate, but the infolding has gone several steps further. The two outer surfaces of the valves not only touch but have become adnate to each other along the new lower suture of the pod, forming a double-walled septum, enclosing a closed though very narrow cavity between the walls. This double-walled partition in some of the segregates may form a narrow stall along the lower suture or extend entirely across the pod, reach the upper suture, and even become adnate to the same as in *Astragalus* proper; or it may be a complete partition, except at the apex, as in *A. Serenoi*. In *Oxytropis* proper, the false partition is formed by the upper suture (the margins of the leaf), which is inflexed; the two margins may stick more or less to each other, but never form a closed cavity.

As there are apparently found all grades from a clearly one-celled pod to a completely 2-celled one in the group it is hard to draw lines, from this condition of the pod alone, between *Phaca*, *Tium*, *Astragalus*, and *Oxytropis*, the 4 oldest genera representing the 4 principal groups; the first with a 1-celled pod without a septum, the second with a partial septum formed by the lower suture, the third with a complete one, and the fourth with one formed by the upper suture.

Most botanists have therefore merged all except *Oxytropis* in *Astragalus*. *Oxytropis* has been kept distinct only on account of one character, the beaked keel, which should not have been regarded as of such importance. It is not customary to base genera on such a condition as whether the leaves are cuspidate or not, and the beak is hardly more than a cusp. Two species undoubtedly belonging to *Hamosa* have beaked keels and some of the species included in *Oxytropis* have no trace of a septum.

If *Amelanchier* and *Gaylussacia* are kept distinct from *Aronia* and *Vaccinium* by a false partition in the carpels formed in a similar way, why should not *Astragalus* be kept separate from *Phaca*? In my opinion *Aronia* is more closely related to *Amelanchier* than to *Pyrus*, of which it often has been regarded as a mere section.

There are also other characters in the pod which should be considered when segregating the genera of the subtribe. The absence or presence of a stipe, or the length of the same, could be considered, but I think it is of little value because we find clearly closely related species in which some have a

sessile and others a long-stipitate pod. I have considered only one case important. In one Alaskan species the pod is born on a distinct gynophore, i.e. the so-called stipe is articulate some distance above the calyx.

The texture of the pod is more important. All grades are found, from a texture thin as paper to a very thick and woody or succulent one. In species closely related otherwise, however, the texture of the pod as a rule is also similar. The general shape in outline as well as in cross-section is very constant in the groups considered by me as genera.

The dehiscence of the pod is very variable in the subtribe, but not in the genera as understood by me. In genera with papery and strongly inflated pods as *Phaca* and *Oxytropis* § *Phacoxytropis* (1-celled), *Atelephragma* and *Phacomene* (partially 2-celled), *Cystium* and *Onix* (2-celled), the pod falls off unopened and rolls around before the wind; or if in the last two genera the pod splits in two through the septum, each half is carried around in the same way. In the genera with thin walled but flattened pods, as *Homalobus* and *Kentrophyta*, the pod is valvate. In those with elongated pods, whether coriaceous, leathery, or woody as *Hamosa* (2-celled), *Tium* (partially 2-celled), and *Lonchophaca* (1-celled) the pod is also valvate throughout. *Contortuplicata* has a pod similar to *Hamosa* but it splits apart very early, forming two false carpels.

In those with shorter and more or less woody pods, as in many species of *Astragalus* (2-celled), *Jonesiella* and *Oxytropis* (partially 2-celled), *Xylophacos*, *Pterophacos*, *Cnemidophacos* (1-celled), the pod is dehiscent valvately at the apex. This mode is exaggerated in *Brachyphragma*, where only the very tip opens, and as the partition does not extend to the apex, the seeds are easily shaken out through the opening. In those genera which have few-seeded thick-walled pods as *Hesperastragalus* (2-celled) and *Orophaca* and *Microphacos* (1-celled), the pod is indehiscent. *Geoprimum* has a very fleshy 2-celled pod, resting on the ground. It splits tardily through the septum; the two halves roll over with the half of the septum as a lid; the seed germinate remaining inside.

I shall treat those genera that have a strictly 1-celled pod first, as this condition must be regarded as primitive, and begin with *Phaca*, which is the oldest one of the group dating back of Linnaeus' Species Plantarum. As botanists have agreed to date the first establishment of modern genera and species from that work, the genus dates back to 1753.

1. PHACOID genera; pod strictly 1-celled

1. *Phaca* L. Sp. Pl. 755. 1753

Perennial or annual herbs, mostly with well developed leafy stems. The leaves are odd-pinnate, the stipules free or united, the leaflets entire. The flowers are borne in axillary racemes. The calyx-tube is campanulate, the lobes 5. The corolla is purple, white, or yellowish; the banner is obovate, almost claw-less and with spreading margins; the wings are usually shorter,

clawed, the blade with a basal auricle; the keel-petals still shorter, the blade more or less lunate, not produced into a beak. The stamens are diadelphous (9 and 1), the sheath straight, the free upper portion of the filaments up-curved. The ovary is sessile or stipitate; the style glabrous, up-curved at the apex, the stigma minute. The pod is papery or rarely membranous, decidedly inflated, sessile or stipitate, mostly rounded-ellipsoid, indehiscent or tardily dehiscent at the apex. The seeds are numerous, mostly obliquely rounded-reniform.

ILLUSTRATION: Plate XLIV A. *Phaca americana* (Hook.) Rydb. $\times \frac{2}{3}$; 1. calyx; 2. banner; 3. wing; 4. keel-petal; 5. stamens; 6. pistil; 7. pod, $\times 1$; 8. pod in cross-section; 9. seed, $\times 2$.

As the type of the genus has been selected *Phaca alpina* L. [= *P. frigida* L.]

SYNONYM:

Physondra Raf. Atl. Journ. 145. 1832. This was based on two species: *Psoralea longifolia* Pursh and *Orobis dispar* Nutt. The first is regarded as the type, as the second does not agree with the diagnosis.

Phaca L. was established on 3 species. As stated above, I regard *P. alpina* L. as the type of the genus, and *P. sibirica* also belongs to it. *P. boetica* has been regarded by some as the type, but I am inclined to exclude it from the genus. *Phaca* is characterized by a papery strongly inflated pod.

The North American species I have divided into three subgenera, *Podocystis*, *Chartocystis*, and *Dermatocystis*. In the two first the pod is of a very thin and papery texture, in the first being more or less stipitate, in the second strictly sessile. In none of the species I have collected have I found an open pod. The pods seemed to be indehiscent, but they may be tardily dehiscent. In herbaria I have found some with a valvate pod, as for instance *P. tricopoda*. In the third subgenus the texture is a little firmer, more like parchment, and here the pod is dehiscent at the apex, and reminds one of that of *Crotalaria*. The subgenus consists of but 7 species, all of western North America except *P. neglecta*, which is found in the northeastern part of the continent.

The genus is the largest of the subtribe and contains 95 North American species, divided into 24 groups. It is also represented by numerous species in the Old World, being next to *Astragalus* proper the largest there, and is the only one of the American genera with a strictly 1-celled pod represented abroad.

2. *Homalobus* Nutt.; T. & G. Fl. N. Am. 1: 350. 1838

Perennial herbs. The leaves are pinnate; sometimes, however, reduced to the terminal leaflets, or the lateral leaflets reduced, linear or filiform, and the terminal one similar to and continuous with the rachis. The flowers are borne in axillary racemes. The calyx is from campanulate to cylindro-campanulate, 5-toothed. The corolla is white to purple; the banner obovate, usually strongly arched and with a very short claw; the blades of the wings obliquely oblanceolate or obovate, with a reflexed basal auricle, about equaling the claw; the keel-petals are shorter, the blade broader and shorter,

from round to acute at the apex. The stamens are diadelphous (9 and 1), the sheath is straight to near the apex, the free portion of the filaments upcurved. The style is glabrous, arched upwards near the apex, the stigma minute. The pod is elliptic to linear in outline, sessile to slender-stipitate, compressed laterally, mostly flat, both sutures being prominent especially when the pod is more turgid, neither of them inflexed or produced into a false partition. The seeds are obliquely reniform, with a deep-seated hilum.

ILLUSTRATION: Plate XLIV B. *Homalobus dispar* Nutt. $\times \frac{2}{3}$; 1. calyx; 2. banner; 3. wing; 4. keel-petal; 5. stamens; 6. pistil; 7. pod; 8. opened pod, with young seeds; 9. pod, in cross-section; 10. seed, $\times 2$.

This genus was established in 1838 by Thomas Nuttall, the most acute of the early American botanists. It was published in Torrey & Gray's Flora of North America. As the type should be regarded *H. dispar* Nutt., which had been originally described, in 1818, as *Orobis dispar*. The pod is thin-walled, as in *Phaca*, but not at all inflated, flat, having valves closely fitting to the seeds, valvate-dehiscent, and with both sutures prominent. In fact, the pod resembles more that of *Lathyrus*, in which *Orobis* now usually is included, than that of *Phaca*.

The genus consists of 59 species, all North American. The writer has divided them into 7 groups. In 4 of these groups the pod is sessile or subsessile, in the other 3 it has a well developed stipe. In one of the latter, represented by *H. collinus* and its relatives, the texture of the valves is firmer, more or less leathery, and the calyx is inclined to be gibbous at the base. The writer has been inclined to remove them from the genus.

3. *Kentrophyta* Nutt. T. & G. Fl. N. Am. 1: 353. 1838

Perennial cespitose herbs, with a woody caudex and diffusely branched stems. The leaves are pinnate, the stipules either scarious and connate high up, or spine-tipped and less connate, the leaflets are 3-7, mostly 5, divergent, linear-subulate or lance-subulate, stiff, strongly ribbed, spine-tipped. The racemes are short, usually 2-flowered. The calyx is campanulate, the 5 lobes subulate, subequal, about as long as the tube. The corolla is ochroleucous or purplish. The banner is obovate or oval, retuse, rather strongly arched. The wings are shorter, falcate, the blade broadly oblanceolate, longer than the claw, with a large reflexed auricle; the keel-petals are still shorter, the blade about equaling the claw, broadly lunate, acutish or obtuse at the apex, auricled at the base. The stamens are diadelphous (9 and 1), the sheath arched above. The pod is ovoid, somewhat compressed, acute at least on the upper suture, few-seeded, indehiscent. The seeds are obliquely reniform, with a deep-seated hilum.

ILLUSTRATION: Plate XLIV C. *Kentrophyta montana* Nutt. 1. habit sketch, $\times \frac{1}{3}$; 2. branch, $\times 1$; 3. calyx; 4. banner; 5. wing; 6. keel-petals; 7. stamens; 8. pistil, $\times 4$; 9. pod; 10. pod, in cross-section; 11. seed, $\times 2$.

This genus was also established by Nuttall in Torrey and Gray's Flora in 1838. It had originally only two species, of which *K. montana* is regarded as the type. The pod is like that of *Homalobus* in being thin-walled, flattened,

and with prominent sutures, but it is few-seeded. The general habit of the plants is very different, the leaflets being narrow and spine-tipped.

It consists of 10 North American species, all natives of the Rocky Mountains, the Great Basin, and its mountain ranges.

4. *Orophaca* (T. & G.) Britton: Britt. & Brown, Ill. Fl. 2: 306. 1897

Densely caespitose, usually pulvinate perennials, with a woody caudex. The branches of the stem are usually very short, densely covered by stipules and old leaf-stalks. The leaves are palmately 3-foliolate, the leaflets oblanceolate to cuneate, entire, silky-canescens. The flowers are borne in small axillary racemes. The calyx is from turbinate-campanulate to cylindric, the teeth subulate, equaling to much shorter than the tube. The corolla is ochroleucous or purplish; the banner is oblanceolate to obovate, tapering into a broad claw; the wings are shorter, the blade obliquely obovate, with a large basal auricle, shorter than the claw; the keel-petals are much shorter, the blade broadly lunate, rounded at the apex. The pod is ovoid or rounded ovoid, leathery, few-seeded, barely equaling the calyx, indehiscent.

ILLUSTRATION: Plate XLIV D. *Orophaca caespitosa* (Nutt.) Rydb. 1. habit sketch, $\times \frac{1}{3}$; 2. branch, $\times \frac{2}{3}$; 3. calyx; 4. banner; 5. wing; 6. keel-petals; 7. stamens; 8. pistil; 9. pod; 10. pod, in cross-section, $\times 1$.

Orophaca was established as a subgenus of *Phaca* in Torrey and Gray's Flora (1: 343) in 1838. The name is credited to Nuttall. It was raised to generic rank by Britton in 1897. *Phaca caespitosa* Nutt. was regarded as the type.

The genus consists of 5 species, all North American and natives of the Rocky Mountain Region. It differs from all the other genera in having digitately 3-foliolate leaves. The pod is small, few seeded indehiscent, not at all inflated, with a leathery, very woolly pericarp. The species are all low, caespitose-pulvinate, and resemble some of the caespitose species of *Trifolium*. The relationship within the subtribe is with *Xylophacos*, but the pod is small and indehiscent, the pericarp close-fitted to the seeds, and the leaves digitately 3-foliolate, not pinnate.

The rest of the published genera with 1-celled pods all have leathery or woody pods, neither strongly inflated nor close-fitted to the seeds. They are all natives of western North America and all proposed by the author, in 1903 and 1905.

5. *Xylophacos* Rydb., Small. Fl. SE U. S. 619. 1903

Perennial herbs, mostly copiously pubescent, usually low, often sub-acaulescent. The leaves are pinnate, the stipules nearly free, the leaflets entire-margined. The flowers are borne in dense, short, sometimes head-like racemes. The calyx is cylindric, the lobes shorter than the tube. The corolla purple, ochroleucous or in one species crimson; the banner is oblanceolate, often retuse at the apex, moderately arched beyond the middle; the wings are shorter, narrow, oblanceolate, with a reflexed basal auricle, scarcely longer than the claw; the keel-petals are shorter, the blade broadly

lunate, rounded at the apex, slightly auricled at the base, shorter than the long claw. The stamens are diadelphous (9 and 1); the sheath has a broad base, and is straight to near the top. The ovary is sessile, the style glabrous, straight to near the apex, the stigma minute. The pod is coriaceous or woody, one-celled, sessile, straight or incurved, strigose or villous, ovoid, lance-ovoid, lance-oblong, or lunate, the lower suture often slightly sulcate, but never producing a false partition. The seeds are numerous, obliquely round-reniform, with a deep-seated hilum.

ILLUSTRATION: Plate XLV E. *Xylophacos Shortianus* (Nutt.) Rydb. $\times \frac{2}{3}$; 1. calyx; 2. banner; 3. wing; 4. keel-petal; 5. stamens; 6. pistil, $\times 1$; 7. pod; 8. pod, in cross-section, $\times \frac{2}{3}$; 9. seed, $\times 2$.

This genus was published in Small's Flora of the Southeastern United States in 1903 and based on two species, of which *Astragalus Shortianus* was regarded as the type. In 1905 (Bull. Torrey Club 32: 661-2) seven more species were transferred to it.

The species are all low, usually decumbent plants, with comparatively large flowers; long calyx-tube, and long-clawed petals. The pod is thick-walled, ovoid or oblong to elongate boat-shaped in outline, tardily dehiscent at the apex, somewhat inflated, *i.e.* the seeds not filling the cavity. Most of the species are canescent, either densely strigose or villous. The genus corresponds nearly to the sections ARGOPHYLLI and ERIOCARPI of Gray's revision of *Astragalus*.

The genus now comprises 46 species, all natives of North America, west of the Missouri River, from the Saskatchewan south to northern Mexico. None are found in the Old World or in Central and South America.

6. *Holcophacos* Rydb.; Small Fl. SE U. S. 618. 1903

Perennial herbs, with a taproot and cespitose caudex. The leaves are odd-pinnate, with many leaflets. The flowers are perfect, borne in short head-like racemes. The calyx is campanulate, the teeth subulate or lanceolate, shorter than the tube. The corolla is lilac, pink or white; the banner is broadly obovate, strongly arched, retuse at the apex; the wings are much shorter than the banner, the blade is oblanceolate, falcate, longer than the claw, and has a reflexed rounded basal auricle; the keel-petals are slightly shorter, the blade, lunate-obovate, about one-third of a circle, longer than the claw. The stamens are diadelphous (9 and 1), the sheath straight to near the apex, the free portion of the filaments arched upwards. The ovary is sessile, glabrous, the style regularly arched upwards, glabrous. The pod is sessile, glabrous, more or less arched, membranous, sulcate on both sutures, but no false partition is formed. The seeds are numerous, obliquely reniform.

ILLUSTRATION: Plate XLV F. *Holcophacos distortus* (Nutt.) Rydb. $\times \frac{2}{3}$; 1. calyx; 2. banner; 3. wing; 4. keel-petal; 5. stamens; 6. pistil, $\times 2$; 7. pod, side-view; 8. pod, dorsal view, $\times \frac{2}{3}$; 9. pod, in cross-section; 10. seed, $\times 2$.

This genus was also established in Small's Flora and based on two species, of which *A. distortus* T. & G. was regarded as the type. In some respects the pod resembles that of *Xylophacos*, being leathery and boat-

shaped in outline, but both sutures are inflexed. There is no trace of a partition, though the sutures approach each other. The general habit of the plants and the flowers resembles more that of some species of *Phaca*, from which it is distinguished by the structure of the pod. The cross-section is nearly that of a couchant ∞ or a pair of spectacles.

The genus contains only the two original species, both natives of the prairie region of central United States.

7. *Cnemidophacos* Rydb. Bull. Torrey Club 32: 663. 1906

Stout perennial herbs, with a cespitose woody caudex. The leaves are pinnate, usually with many narrow leaflets; the stipules are usually slightly united with petioles, but strongly connate around the stem. The flowers are perfect, mostly borne in strict many-flowered racemes. The calyx is campanulate, the teeth linear-lanceolate, mostly equaling the tube in length. The corolla is yellow, ochroleucous, white, or purplish; the banner is obovate, tapering at the base, often retuse at the apex, only slightly arched; the wings are shorter, the blade oblanceolate, usually with a rounded auricle, longer than the claw; the keel-petals are broader and shorter, the blade obliquely obovate, or semi-orbicular, with a reflexed auricle, shorter than the claw. The stamens are diadelphous (9 and 1); the sheath straight to near the oblique apex, the free portion of the filaments arched upward. The ovary is sessile, the style curved towards the apex, the stigma minute. The pod is one-celled, coriaceous, sessile, ovoid or ellipsoid in outline, often thicker than wide (*i.e.* the diameter from center to center of the valves greater than the distance between the sutures), rounded at the base, abruptly contracted at the apex, with both sutures rather prominent. The seeds are numerous, obliquely reniform, with a deep-seated hilum.

ILLUSTRATION: Plate XLV G. *Cnemidophacos flavus* (Nutt.) Rydb. $\times \frac{2}{3}$; 1. calyx; 2. banner; 3. wing; 4. keel-petal; 5. stamens; 6. pistil, $\times 2$; 7. pods; 8. pod, laid open, $\times 1$; 9. pod, in cross-section; 10. seed, $\times 2$.

The genus was based on *Astragalus flavus* Nutt.

SYNONYM:

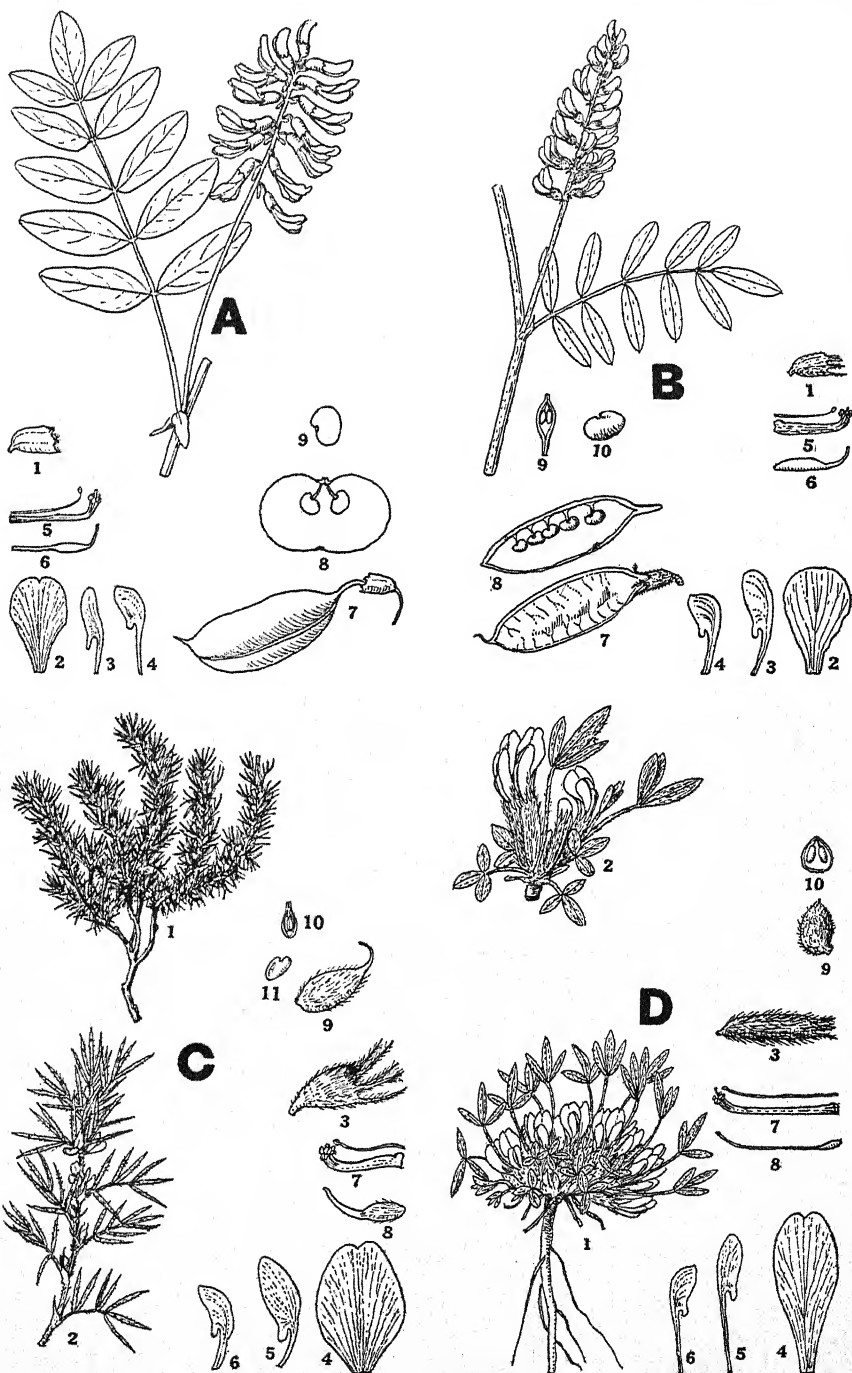
Ctenophyllum Rydb. Bull. Torrey Club, 32: 663. 1906. This genus, based on *Phaca pectinatus* Hook., should probably be included in *Ctenophyllum*.

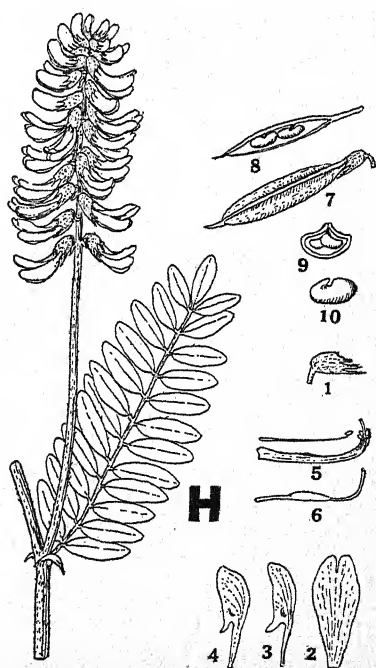
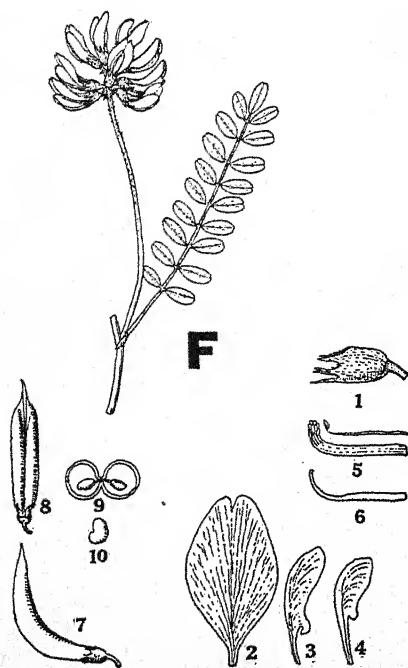
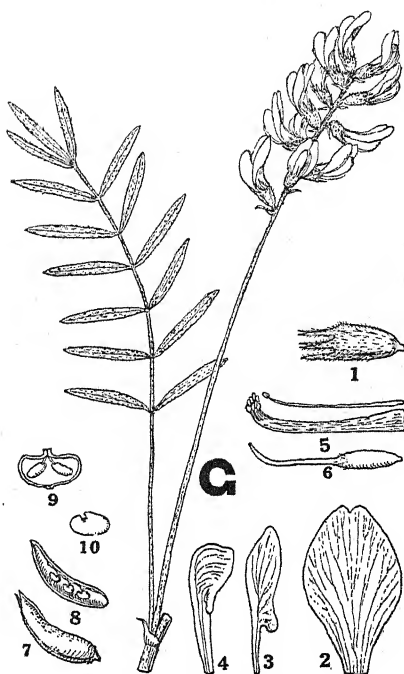
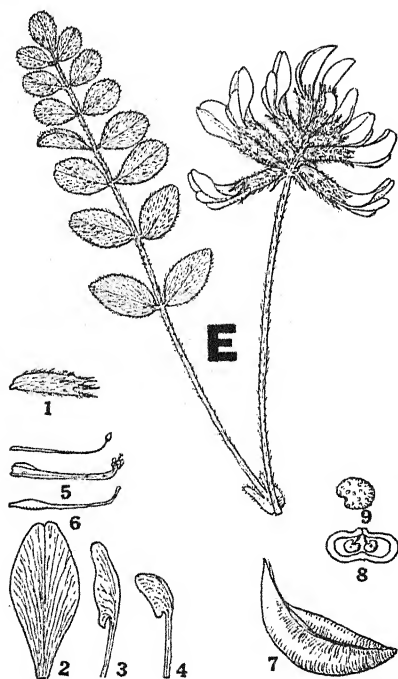
The pod in the genus is short, leathery or woody, ovoid in outline, turgid, sometimes slightly sulcate on the lower suture, but without a partition, the upper suture or both prominent, 2-valved at the apex. The plants are erect but rather low, with usually ochroleucous corollas.

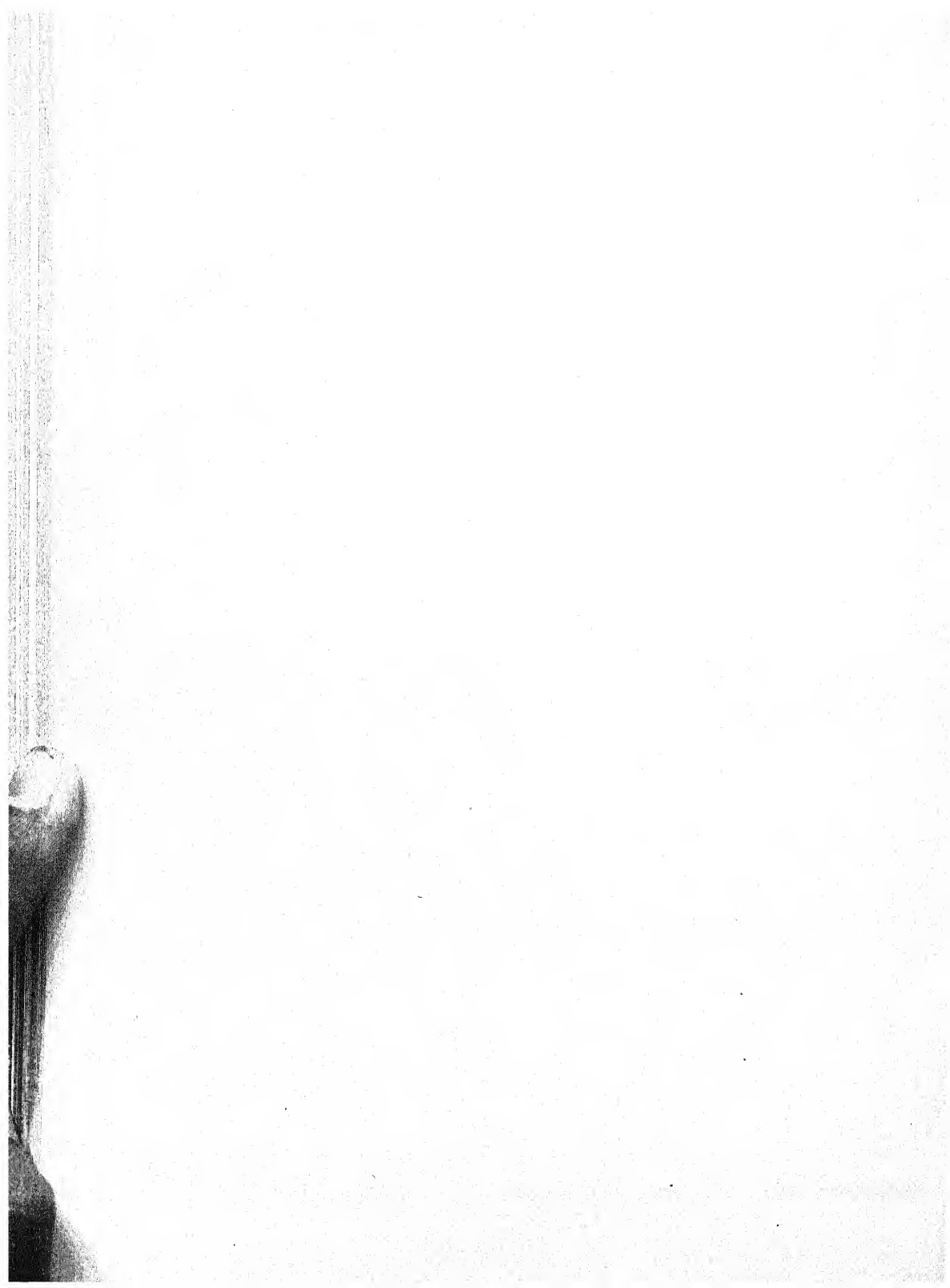
The genus consists of 15 species, all from the arid regions between the Rocky Mountains and the Cascades and Sierra Nevada except one, from the Great Plains east of the Rockies.

8. *Diholcos* Rydb. Bull. Torrey Club 32: 664. 1906

Stout perennials, usually forming clumps. The leaves are pinnate, with many thick leaflets, and free stipules. The flowers are numerous, borne in dense, spike-like racemes. The calyx is campanulate, gibbous at the base on the upper side, the 5 teeth shorter than the tube, unequal,







subulate, the lowest the longest. The corolla is purple, white or ochroleucous; the banner is oblanceolate, or narrowly obovate, retuse, gradually arched, clawless: the wings are about equaling the banner, the blade obliquely oblanceolate, with a prominent, acute basal auricle; the keel-petals are similar but broader, the blade broadly lunate. The stamens are diadelphous (9 and 1), the sheath arched above the middle. The ovary is stipitate, the style glabrous, arched throughout, the stigma minute. The pod is coriaceous, stipitate, the body oblong, straight, rounded on the lower suture, deeply 2-grooved above, the upper suture prominent. The seeds are 6-20, obliquely reniform, with a deep-seated hilum.

ILLUSTRATION: Plate XLV *H. Diholcos bisulcatus* (Dougl.) Rydb. $\times \frac{2}{3}$; 1. calyx; 2. banner; 3. wing; 4. keel-petals; 5. stamens; 6. pistil; 7. pod, dorsal view; 8. pod, laid open, $\times 1$; 9. pod, in cross section; 10. seed, $\times 2$.

The genus was established at the same time as the preceeding genus, *A. bisulcatus* Dougl. being the type. In habit, the species resemble much those of the preceeding genus; the pod is also similar except that it is stipitate and has a trough-like groove on each side of the acute upper suture.

It consists of 6 species, from the Great Plains and Great Basin regions of Western United States.

NEW YORK BOTANICAL GARDEN

THE PHYTOPLANKTON OF SOME ARIZONA POOLS AND LAKES

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INTRODUCTION

A few years ago the second author (H. S. Colton) planned a study of the Entomostraca in the zoöplankton of the water holes and streams in Coconino County, Arizona, of which Flagstaff is the county seat. Collections were made during July-September 1923 and 1925, to the number of about 100 samples. Analysis of these from the original viewpoint never having been completed, he placed the material in the hands of the first author for a study of the algae present. An inspection of the samples was made in 1926 and more extensively in 1928. Responsibility for statements regarding the identity of the plants reported, and in general for outlining the botanical phase of this study rests with the first author, while his associate has contributed the material and field records, and the indispensable information regarding localities and their environments.

Arizona is practically unknown so far as its algal flora is concerned. The fact that a considerable portion of the state is exposed to seasonal drought tends to limit to an extent the character of the algae of smaller depressions which dry out in the spring, and under such conditions the ephemeral water courses likewise are not generally rich in algae. However, there is no reason to doubt that from the larger lakes and the alpine districts where there is more constant moisture, a fairly considerable variety of algae may be expected. Excepting a very few lists of diatoms we know as yet practically nothing of this flora, which from the severity of climatic conditions is bound to present an interesting aspect. In the strictly alpine regions no very peculiar general features are to be expected, for the aspect of alpine algal floras is very similar through the northern hemisphere (11). It is in the plateau and mesa districts, and also in the lowlands, that the special conditions and specialized floras are to be expected. Truly alpine conditions at this latitude are expected to appear at about 11,000 feet, so that the material for this study represents a distinctly sub-alpine zone, even though a rather extensive altitudinal range is included (3,500-8,800 ft.), contrasting with parts of British Columbia where alpine conditions appear at 7,000 ft. (11).

From neighboring districts there is very little in the way of lists of algae to compare with the present. Robbins' list (8) of the freshwater algae of Colorado is the best of these, especially because it covers an equally wide range of altitude. We find that the only group in which there is a large

proportion of species listed from both districts is that of the Protococcales, which has many ubiquitous members. The desmids of Wailes' alpine Colorado stations (12) represent a considerably higher altitude range (11,660–12,740 ft.), and a close correspondence of floras can not be expected. However, almost all of those noted from the Arizona stations have been reported from elsewhere in North America. Schmidle (10) gives a brief list of algae from about Denver (Colorado) which includes only 3 non-desmids, all cosmopolitan, and only *Pandorina* and a *Cosmarium* appearing also in the Flagstaff area. Cushman's southwestern Colorado list (3) and Cockerell's Colorado list (2) also show little similarity. In undertaking a study of this Arizona material it was hoped that it would connect directly with the studies made by the first author over a considerable period in British Columbia (11), and some similarity, indeed, did appear. This was particularly marked in the widely ranging Protococcales, and the more markedly infra-alpine British Columbia flora (3,000–4,500 ft.) agreed tolerably with that of Arizona. Since the latter was of plankton algae with benthic species accidentally present, while the northern study was of shore and bottom material with the plankton types accidentally present, too great a coincidence must not be expected. Probably the most interesting novelties are the species of *Characium* (or similar genera) growing attached to plankton animals, for they seem largely new and increase to a proportionally notable degree the number of these plants of curious habitat known from America.

Coconino County in north central Arizona—one of the largest counties in the United States (225 miles long and 160 miles wide)—presents a great variety of natural conditions, yet throughout the area there are certain peculiarities which give northern Arizona a characteristic aspect. It is a region of high plateaus, deep canyons, and flat-topped mesas, dominated by the great San Francisco volcanic field with extensive lava flows and numberless craters. The differences in altitude, from 2,500 to 12,740 feet, with associated differences in temperature and rainfall, and the varying conditions due to underlying rock (sandstone, shale, limestone, basic or acid lava) all present ecological factors which may affect the flora and fauna.

The Colorado River in the Grand Canyon drains the northern part of the county. Its principal tributary, the intermittent Little Colorado, drains the eastern side, while several streams, Sycamore Creek (intermittent), Oak Creek, Beaver Creek, Clear Creek, and Fossil Creek collect the water from the southern and western area. These latter streams are tributaries of the Verde River and so belong to the Gila drainage system.

North and west of the Colorado River lies the extensive limestone Kaibab Plateau; south of the Grand Canyon the limestone Coconino Plateau merges into the San Francisco Volcanic Field; and variegated sandstones and shales overlie the limestone in the Valley of the Little Colorado, giving the name to the "Painted Desert."

Although much of the area receives an ample rainfall of 25 inches, the porous limestone or lava soil absorbs most of the water, wherefore living streams are very few. The sandstone and shale areas are mostly low in altitude, receiving little precipitation (5-10 in.). Almost all the water over this large area, except in a pitifully small number of springs, lies in natural or artificial pools, called tanks from the Spanish word for ponds ("tanques"). These range in size from puddles to lakes five miles long, and except in times of flood they have neither inflow nor outflow. These water holes form the habitat for a rich entomostracan fauna dependent upon a phytoplankton which is described below. At various stations over the plateau samples of the plankton were collected by means of a plankton net of no. 12 bolting cloth, and the material was preserved in 4 percent formaldehyd.

DISCUSSION OF STATIONS

The stations in this study have been grouped into certain geographical regions:

- I. The Kaibab Plateau.
- II. The Painted Desert.
- III. The San Francisco Volcanic Field, which includes: (1) The drainage of Lower Walnut Creek (intermittent); (2) The drainage of Upper Walnut Creek (intermittent) and the Lake Region of Arizona; (3) The San Francisco Peaks; (4) Chavez Pass; (5) Plateau east of Oak Creek Canyon; (6) Plateau south of Bill Williams Mountain.
- IV. The Verde Drainage.

I. Kaibab Plateau

(a) Pond or tank in limestone northeast of the Bright Angel Ranger Station (66). About 50 feet in diameter, seemingly permanent, and at an elevation of 8,500 feet. A rather varied sample, the crustacea bearing 2 species of *Characium*. Algae dominated by *Spirogyra* (2 species) and *Desmidium*, associated with *Staurastrum*, *Closterium* (3 species), *Oedogonium*, *Scenedesmus*, and *Dictyosphaerium*.

(b) V. T. Park, named after a cattle company. A pond in a limestone sink, of about 150 feet diameter, a watering place for cattle and deer. It lies about a mile south of Lodge on the west side of the road and seems to be permanent; altitude 9,000 feet. Crustacea were prominent in the sample. The algae were dominated by *Spirogyra*, but there were remnants of flagellates, *Desmidium*, *Eudorina*, and a species resembling *Askenasyella*.

(c) House Rock Valley. An artificial tank of the Grand Canyon Cattle Company, supplied by running water from a spring. A watering place for cattle about 200 feet in diameter at an altitude of 5,200 feet. The phytoplankton was dominated by *Microcystis incerta* and *Anabaena spirioides* fa., associated with *Actinastrum*, *Scenedesmus*, and *Ankistrodesmus*.

(d) Jacobs Lake. An artificial tank about 300 feet in diameter. Prob-

ably permanent, a watering place for cattle at an altitude of about 7,200 feet. The sample contained a considerable proportion of silt and crustacea. The phytoplankton was very poor, of rather frequent *Oscillatoria* and *Desmidiium*.

II. Painted Desert

(a) Roden's Spring. Samples from two sources: an artificial pool in red sandstone about 50 feet in diameter, and a 10-foot iron tank. Both are fed by water from a spring, and used in the winter for watering cattle; altitude 4,500 feet. In the pool there were some crustacea accompanied by a copious deposit of red mud. On the animals were specimens of *Characium*, and rare coenobia of *Pediastrum* were seen. In the iron tank there was an exceedingly scanty phytoplankton of *Selenastrum*, *Scenedesmus*, *Oocystis*, and minor items.

(b) Tank. An artificial tank about 200 feet across, two and a half miles southeast of "Two Guns," a trading post on Highway 66 at Canyon Diablo, in sandstone. Probably dry at the end of June; altitude 5,500 feet. Plankton of crustacea abundant, associated with a few Peridineae.

III. San Francisco Volcanic Field

1. Lower Walnut Creek Drainage.

(a) Walnut Tank. This lies in the bottom of a basaltic canyon of the intermittent Walnut Creek. When visited the water was confined to certain deep holes about 2 feet in diameter; the altitude was 5,500 feet. With a few tadpoles and *Apus* and other crustacea there was an interesting if sparse phytoplankton. The genera represented were: *Scenedesmus*, *Coelastrum*, *Oocystis*, *Tetraedron*, *Lagerheimia*, *Pediastrum*, and a few diatoms.

(b) Jack LeBarron's Ranch. An artificial pond, about 300 feet across, on limestone and volcanic ash. Used for watering sheep, it usually contains water; the altitude is about 5,600 feet. The plankton consisted mainly of crustacea, but there were a fair number of diatoms.

(c) Grass Tank. Three natural pools about 10 by 30 feet in a basaltic canyon, situated 3 miles north of the Piper Ranch. The water seems to be permanent; altitude 5,800 feet. In bulk dominated by crustacea, including *Apus*, often with abundant euglenoids. The pools had the same general type of flora, which was rich in variety of Chlorophyceae, and included the following genera: *Aphanizomenon*, *Phacus*, *Closterium*, *Cosmarium*, *Selenastrum*, *Coelastrum*, *Tetraedron*, *Scenedesmus*, *Elakatothrix*, *Ankistrodesmus*, *Oocystis*, *Schroederia*, *Dictyosphaerium*, *Actinastrum*, *Pediastrum*, *Oedogonium*, and *Ophiocytium*.

(d) Turkey Tanks. These are 2 natural pools in a basaltic canyon of Walnut Creek. The largest is over 6 feet deep and about 15 by 30 feet in diameters. It is not known to dry up, and stock can approach it at one point over a very rough trail; the altitude 6,000 feet. The samples showed

fine silt and a few crustacea, a fair number of diatoms, *Aphanizomenon*, a few euglenoids, fragments of *Oedogonium*, and fruiting *Spirogyra Weberi*.

(e) Power's Tank. This lies southeast of Doney Park in the Rio de Flag, and is an artificial tank about 50 feet in diameter in a basaltic canyon. It is used for watering sheep, but its permanency is unknown; the altitude 6,500 feet. The sample consisted mostly of sand, fine silt, and crustacea; small diatoms are scarce, euglenoids frequent; fragments of *Spirogyra*, *Selenastrum*, *Actinastrum*, and *Ankistrodesmus* also present.

(f) Turkey Hills. On the mesa east of South Crater, an artificial tank about 50 feet in diameter in a basaltic canyon at 6,700 ft; used for watering sheep, its permanency is unknown. The plankton catch yielded silt and sand with *Eudorina*.

(g) Turkey Hills Gap. An artificial tank about 30 feet in diameter in volcanic ash. Its permanency is unknown, but it is used for watering sheep; altitude 6,900 feet. In addition to silt, sand, and crustacea the plankton yielded a few *Pinnularia* and filaments of *Schizothrix*.

(h) Turkey Hills. This was an artificial tank about 30 feet in diameter on limestone among much volcanic ash southeast across the Santa Fe Railroad at 6,800 ft. It is used for watering sheep. The plankton showed fine silt, pollen, *Volvox*, *Gonium*, and *Eudorina*.

(i) Walnut Canyon Road. Two miles southeast of Bottomless Pits; this series is from an artificial tank which is at the right of the road in a limestone canyon at 6,700 feet and which usually contains water. The yield was of sand, silt, and vegetable fragments, with few crustacea, very few small diatoms, fragments of slender *Oscillatoria* and *Oocystis*.

2. The Lake Region and Upper Walnut Creek.

(a) Lake Mary. An artificial, permanent lake without regular inflow or outflow, 5 miles long and from one-fourth to one-half mile wide, lying in a limestone valley at an altitude of 6,800 feet. There was little difference in flora between the deep and the shallow ends. In 1923 *Aphanizomenon* in a waterbloom dominated the flora, associated with diatoms, *Botryococcus*, *Anabaena*, and *Microcystis*. In 1925 the sample showed many crustacea and a very rich algal content dominated equally by *Eudorina* and *Volvox*, with abundant *Botryococcus* and *Peridinium*, the minor items being *Aphanizomenon*, *Gonium*, *Cosmarium*, and *Staurastrum*.

(b) Marshall Lake. A shallow permanent natural lake about a mile long and half as wide on a basaltic plateau, without regular inflow or outflow, at an altitude of 7,100 feet and used for watering sheep and cattle. *Aphanizomenon* in a waterbloom dominated the flora, associated with diatoms, *Botryococcus*, and *Microcystis*.

(c) Vail Lake. A shallow, permanent natural lake about a half mile in diameter on a basaltic mesa without regular inflow or outflow. It is a watering place for sheep and cattle at an altitude of 7,100 feet. In 1923 it was reported that cattle and sheep had died from drinking the water. It

had a dense waterbloom of *Microcystis*, which lined the shores in dense windrows. The lake also teemed with the water dog, *Amblystomum tigrinum*: the dead bodies of many were partly buried in the windrows of algae. In 1926 the conditions were normal. The plankton was dominated by a waterbloom of *Microcystis aeruginosa*, associated with diatoms, *Anabaena*, and minor items; the crustacea were fairly abundant.

(d) Mormon Lake. This, the largest natural body of water in Arizona, is 5 miles long and 3 miles wide, lies at an altitude of 7,000 feet and is well stocked with fish. However, it was dry in the early nineties and the early years of the present century. The sample was taken by boat from the west side. The phytoplankton was dominated by a waterbloom of *Aphanizomenon*, associated with *Anabaena* and *Pandorina*.

3. *San Francisco Peaks*. The following stations girdle the peaks:

(a) Lockett's Garden. An artificial tank about 75 feet across, fed by springs in a basaltic valley at an altitude of 7,200 feet. This is permanent, and a watering place for stock and sheep. The sample was mainly of silt and crustacea, with a very poor phytoplankton of diatoms and *Scenedesmus*.

(b) Bismark Lake. A natural pool on the west slope of Mt. Humphrey, about 100 yards in diameter and at an altitude of 8,800 feet. It is a watering place for numerous cattle, but frequently dries up, as in 1925 and 1926, and it was reduced to a mud puddle in 1927. The vials yielded crustacea as a prominent part of all samples. The phytoplankton was mainly of diatoms, with occasional cells of other groups, but altogether very poor.

(c) Walker Lake. A natural lake in the crater of a volcano and about 200 yards in diameter, this is not known to go dry. Its altitude is 8,300 feet and it serves as a permanent watering place for cattle. The sample was quite rich, dominated by *Peridinium* with *Dinobryon*, *Microcystis*, *Tolypothrix*, *Volvox*, *Closterium*, *Pleurotaenium*, *Micrasterias*, *Mougeotia*, *Characium*, *Oedogonium*, and *Ophiocytium*.

(d) Kelley Tank. On the northwest slopes of the mountains, in a valley among cinder cones at an altitude of 7,700 feet, lies this watering place for cattle. Having been empty in July, this station showed only a few diatoms among the silt and pollen grains when sampled in August.

(e) Dead Man's Ranger Station. A small pool in a basaltic canyon north of the mountains at an altitude of 6,600 feet, probably not permanent. Crustacea dominate this sample, with associated *Characium*, *Phacus*, *Closterium*, and a few diatoms.

(f) Jack Smith's Tank. An artificial pond in volcanic debris on the east side of the mountains at 7,300 feet used mainly for watering sheep. It is about 100 feet in diameter and is often dry in the spring. Abundant crustacea monopolizing this sample were heavily loaded with species of *Characium*.

4. Chavez Pass Region.

(a) Chavez Pass. This is a break in the escarpment through which the pioneer road between Winslow and Prescott once entered the lava fields. Two artificial tanks in the red sandstone, about 200 feet in diameter, lying below the pass at an altitude of 6,300 feet were sampled. They are used for watering sheep. The plankton samples were scanty, yielding a few diatoms, *Phacus*, *Oocystis*, *Ankistrodesmus*, and *Oscillatoria* with abundant silt.

(b) Harlow Yeager's Sheep Ranch. An artificial tank about 200 feet in diameter 3 miles south of Chavez Pass at 5,800 feet. The sample contained silt and some sand, associated with frequent reproducing *Eudorina*.

5. Plateau East of Oak Creek.

(a) Mund's Park. An artificial tank on the lava plateau in an arroya at an altitude of 6,400 feet, used for watering stock and apparently permanent. Very many small crustacea here, the phytoplankton dominated by *Volvox aureus*, with *Eudorina* and minor items.

6. Plateau South of Williams.

(a) Artificial Tank. Located 7 miles to the south of Williams at 7,000 feet, showing abundant crustacea, with silt but no algae.

(b) J. D. Dam. An artificial lake about one-fourth mile in diameter at an altitude of 6,500 feet. *Aphanizomenon* and *Melosira* were occasional, associated with *Desmidium* and minor elements.

IV. Verde Drainage

Oak Creek is a trout stream originating in a series of springs and flowing out of the plateau through a deep, picturesque canyon.

(a) Oak Creek, above Oak Creek Lodge. Stream about 20 feet wide and clear at 5,900 feet. The samples were mostly sand and silt with numerous small diatoms, and the associated algae were dominated by *Spirogyra* (2 species) with *Oscillatoria*, *Calothrix*, *Nostoc*, *Stigeoclonium*, and *Scenedesmus*.

(b) Oak Creek, at Sidiña. Stream about 30 feet wide, not very clear, at 4,500 ft. Samples were mostly silt, with small diatoms. The associated algae were *Nodularia*, *Oscillatoria*, *Closterium*, *Spirogyra*, *Ankistrodesmus*, *Scenedesmus*, *Ulothrix*, and *Stigeoclonium*.

(c) A transient roadside tank, halfway between Cottonwood and Camp Verde, the only station not in Coconino County. The rock is limestone, the altitude 3,000 feet. This station contains water after rains, but is dry most of the time. The samples showed many crustacea, with some sand and silt, and phytoplankton represented by a few *Closterium* cells.

SUMMARY OF ECOLOGICAL CONDITIONS

This study suffers from the fact that the samples do not show repeated collections at each locality over a term of years, there frequently being only one or two from a given locality. In about 100 samples some 30 localities are represented, and the range of ecological conditions is too great to enable one

to assume that all are entirely adequately investigated. However, in a pioneer treatment such as this, dealing with a rather difficult type of country, even as extensive an assortment of material as the present is somewhat of an accomplishment and when one considers the comparative scarcity of bodies of standing water it may be taken as fairly representative of the territory.

The collections in hand show examples of the phenomenon of "water-bloom," but for the most part they quite appropriately exhibit a variety of types present in a moderate or sparse total volume. To pretend to offer valid generalizations respecting phytoplankton based on single collections from a considerable number of localities is dangerous because the "flowering" of bodies of water is well known to be an ephemeral phenomenon, likely to occur at certain seasons, but generally of short duration. On such flowerings depend the reports of vast abundance of small algae that, as "water-bloom" of one species or another, interrupt the more gradual seasonal cycle of the algal populations, and obscure the more characteristic flora.

A consideration of the distribution of the samples from the standpoint of altitude does not show any particular correlation with "water-bloom" either in kind or abundance. Between 3,500 and 5,000 feet there were 9 samples from 4 localities, without notable features. Between 5,000 and 7,000 feet there were 41 samples from 23 localities, one of which (in House Rock Valley) showed a bloom of *Microcystis incerta* with *Anabaena spirioides* fa., three showed a fairly varied flora of mixed Chlorophyceae, Mormon Lake a bloom of *Aphanizomenon flos-aquae*, and Munds Park with Lake Mary a bloom of *Volvox aureus* and *Eudorina elegans*. Between 7,000 and 8,000 feet there were 13 samples from 5 localities, of which Marshall Lake showed a bloom of *Aphanizomenon flos-aquae*, Vail Lake one of *Microcystis aeruginosa*, and another a fairly varied flora of mixed Chlorophyceae. Finally, at elevations above 8,000 feet 9 samples came from 5 localities, of which Kelly Tank showed a bloom of *Volvox aureus*, *Eudorina elegans*, and *Peridinium*, while a tank at Bright Angel Ranger Station showed a varied flora of Chlorophyceae.

Considered from the standpoint of the character of the flora apart from water-bloom, together with the history of the body of water—an artificial or natural origin—certain observations are possible. Nine classes of samples are convenient, in 3 divisions, as shown in table 1.

As the number of samples taken was affected by the apparent richness of the localities it is better to take the latter for comparison, especially as this reduces somewhat the slight although almost unavoidable overlapping of records. Natural tanks and lakes are much more likely to have a flora rich in algae than artificial ones (18 : 10), and less likely to be barren of phytoplankton (6 : 9). A varied flora of Chlorophyceae or of Bacillarieae is practically limited to natural bodies of water (9 : 1), for the exception is a pool in which water-lilies have been introduced, and they would be very

likely to bring with them a variety of small algae and to establish them in their new quarters. It would appear that Myxophyceae were not particular in this respect, and Volvocales prefer artificial pools, though the number of localities showing these were few. Myxophyceae and Volvocales appeared much more likely to dominate the flora and to form water-blooms than others among the groups present in fair abundance (8 out of 10 localities contrasted with 3 out of 18).

TABLE I

Class	Samples	Localities	Natural	Artificial
I. Practically free from algae; Crustacea etc., scarce.....	7	5	2	3
II. Practically free from algae; Crustacea etc., numerous.....	11	10	4	6
III. Dominant algae:				
Myxophyceae, always as waterbloom.....	17	5	3	2
Volvocales.....	5	5	1	4
Euglenoid flagellates.....	1	1	1	—
Peridinium.....	3	3	1	2
Filamentous Conjugales.....	4	3	2	1
Chlorophyceae, rich mixed.....	6	3	2	1
Bacillarieae frequent.....	10	7	7	—

While the altitude and the cultural history of the lakes may be expected to afford information accounting for some phases of the distribution of these algae, perhaps it is more accurate to consider the samples from the standpoint of the type of vegetation or landscape aspect in which the lakes or pools are situated, and for many of them it is possible to do this. From the Upper Mountain Forest zone (lower portion—Canadian Zone of Merriam) there were 5 samples of which 3 (Crater Lake, Bright Angel tank, and Walker Lake) showed a varied flora, the second rich in Conjugales and the last in Peridineae. From the Lower Mountain Forest (Transition Zone of Merriam) in the immediate vicinity of Flagstaff 5 samples (Lockett's Gardens, Powers Tank, Walnut Canyon Road, Lake Mary Road) all showed very sparse vegetation, except for frequent Euglenas in the second and last named. At a somewhat greater distance to the south samples from Lake Mary, Marshall Lake, Mormon Lake, and Munds Park were studied, the first 3 with abundant vegetation, principally of *Aphanizomenon*, the last of *Volvox* with *Eudorina*. To the north of the city samples came from Bismark Lake and the Jacobs Lake vicinity, all without important algal constituents. From the Xerophytic Forest area (Upper Sonoran Zone, Forest Phase of Merriam) 6 samples were recorded (Chavez Pass, south of Yeager's Ranch, Turkey, Grass, and Deadman's Ranger Station tanks, Oak Creek near Lodge), which showed a wide range of plant populations, mostly sparse, but the second with frequent *Eudorina*, the fourth with abundant euglenoids and the last with abundant spirogyras. The Semi-Desert area (Upper Sonoran Zone, Grassland Phase of Merriam) is represented by 5

samples also (Oak Creek at Sidonia, near Winslow road, Jack LeBarron's, Walnut, and Roden's Spring Mud tanks) comparatively near Flagstaff, and another (House Rock Valley) much further to the north. Only Walnut Tank with a variety of Protococcales and the last station with *Microcystis* and *Anabaena* showed marked algal character. Finally, one sample from the Desert Area itself (Lower Sonoran Zone of Merriam) in the Verde Valley showed a very sparse flora of *Closterium* alone.

Similarly, a portion of the samples are practicably classified on the basis of country rock. From acid lava in the form of dacite, rhyolite, or related substance only 2 samples are available, one (Bismark Lake) being very barren, the other (Mormon Lake, west side) being covered with a bloom of *Aphanizomenon*. Basic lava, principally in basalt formations, gave a much larger number of samples (20) from Turkey and Powers tanks, gap in Turkey Hills, Crater, Vail, and Marshall Lakes, Grass, Walnut, and Jack LeBarron's Tanks, Lockett's Gardens, Bismark Lake, Deadman's Ranger Station tank, Mormon Lake (east side), and Walker Lake. Of these the Powers and Grass tanks showed frequent or abundant euglenoids, the Marshall Lake a bloom of *Aphanizomenon*, the Grass and Walnut Tanks a varied flora of Protococcales and the last was dominated by Peridinales, but the samples were not otherwise rich or characteristic. Limestone formations contributed 7 samples (Walnut Canyon road, Lake Mary, House Rock Valley, Bright Angel Ranger Station tank, Jacobs Lake and vicinity, tank near Lake Mary road, and another in Verde Valley) of which the second showed a bloom of *Aphanizomenon*, the third one of *Microcystis* and *Anabaena*, the fourth was rich in a variety of Conjugales, the penultimate in abundant euglenoids, so that while there was not a fixed type of flora there was a higher proportion of well populated pools. Finally, from sandstone and shale as environments there were 6 samples (Chavez Pass, Roden's Spring Mud Tank, Oak Creek at Sidonia and above Lodge) all very poorly populated except the penultimate, where *Spirogyra* afforded a moderate quantity of vegetation.

SYSTEMATIC LIST OF SPECIES

In the following list are included those plants which seemed fairly certain in determination. The writer has ventured to include the desmids, since there were comparatively few species involved, but the diatoms have been omitted, for they were usually very scarce and the labor of preparing the samples for a set of diatom determinations would hardly have been repaid. Generally the species present were minute Naviculæ, but occasional filaments of *Melosira* were seen, *Nitzschia*, *Amphora*, and *Cymbella* were occasional, and in a few samples *Cymatopleura* was quite abundant.

There are a few other items that were omitted from the list, but deserving of mention. In a very considerable number of the samples there were from a few to an important number of euglenoid flagellates present, but viewed in

these formalin-preserved samples it was impossible to give species names to them. Usually the important cases of this kind have been mentioned in the description of the several lakes. In a few samples there were minute tufts of filaments in arrangement suggesting a well-developed *Dichothrix*, but no clear understanding of their nature was to be had. In Chavez Pass tank, Crater Lake, and Roden's Spring there was an abundance of very tiny chlorophyceous cells, spherical, but without definite enough features to serve for determination in the preserved state.

MYXOPHYCEAE

ANABAENA CIRCINALIS (Kuetzing) Rabenhorst. Near Turkey Hills, Mary's Lake (in part doubtful), Marshall Lake; 6,800–7,100 ft.

ANABAENA LEMMERMANNII P. Richter. Mormon Lake; at 7,000 ft.

ANABAENA SPIROIDES Lemmerman. G. C. Cattle Co. tank in House Rock Valley at 5,200 ft. Cells of the spore-forming filaments becoming rather long, perhaps in relation to deferred spore transformation.

ANABAENA sp? Mormon Lake, at 7,000 ft. Filaments 3.8μ in diameter, curved; spore long oval, distinctly tapering towards the ends which are truncate, $7.6 \mu \times 23 \mu$; heterocysts $4 \mu \times 14 \mu$.

APHANIZOMENON FLOS-AQUAE (Lyngbye) Ralfs. Frequent, often forming a waterbloom. Turkey Tanks, Power Tank, Mary's Lake, Marshall Lake, Grass Tank, J. P. Dam, south of Willows; 5,800–7,100 ft.

CALOTHRIX PARIETINA (Naegeli) Thuret. Rare fragments. Iron Tank at Roden's Spring, Oak Creek above Lodge; 4,500–5,900 ft.

CHAMAESIPHON sp? Grass Tank; at 5,800 ft. On crustacea; length 12.6μ , diameter 2.1μ , no foot or sheathing membranes visible, fairly refractive, straight to moderately curved, cylindrical with rounded ends. As no methods of spore formation were observed in any of the 50–100 individuals noted, it seems quite unsafe to give a specific name to this plant, which shows some points in resemblance to *C. cylindricum* Boye-Petersen from Iceland.

MICROCYSTIS AERUGINOSA Kuetzing, fa. *occidentalis* W. R. Taylor n. fa. Occasional, and at times forming a waterbloom. Mary's Lake, Marshall Lake, Grass Tank, Vail Lake, Walker Lake; 5,800–8,300 ft. Plant as in the typical species, but the cells 4.7 – 5.7μ in diameter, and perhaps not to be distinguished from the var. *major* Witttr. (5 – 6.5μ) collected near Königgrätz in Bohemia.

MICROCYSTIS FLOS-AQUAE (Wittrock) Kirchner? Doubtful record, being perhaps based on unclathrate colonies of *M. aeruginosa* fa. Mary's Lake, Marshall Lake; 6,800–7,100 ft.

MICROCYSTIS INCERTA Lemmermann. Forming a major part of a mixed waterbloom, House Rock Valley; 5,200 ft.

NODULARIA SPUMIGENA GENUINA Bornet & Flahault. Oak Creek at Sidonia; 4,500 ft.

OSCILLATORIA SANCTA Kuetzing. Oak Creek, at Sidonia and also above Lodge; 4,500–5,900 ft.

OSCILLATORIA sps. Undeterminable specimens of *Oscillatoria* occurred very sparingly in 7 samples. Measurements would suggest that probably 3 forms were represented, one slender and reaching $2\ \mu$ in diameter, another $3.8\text{--}5\ \mu$ in diameter, a third $32\ \mu$.

PHORMIDIUM sp. This genus, hardly to be expected except as an accidental element in plankton samples, was once found, the filaments $0.75\ \mu$ in diameter, scanty and indeterminable.

TOLYPOTHRIX LANATA Wartmann. Fragments only, in Walker Lake at 8,300 ft.

FLAGELLATAE

DINOBRYON UTRICULUS Stein. Few, in Walker Lake at 8,300 ft.

PERIDINIUM sp. A representative of this genus very important in Walker Lake, both in the active and the encysted stages; 8,300 ft. Other samples occasionally contained a few Peridineae, but determinations were not effected.

PHACUS LONGICAUDA (Ehrenberg) Dujardin. Grass Tank; 5,800 ft. rare.

PHACUS PLEURONECTES (O. F. Muller) Dujardin. Chavez Pass (6,300 ft.), at and near Jacob's Lake, Pot-hole, at Deadman's Ranger Station; 6,300–7,200 ft.

CHLOROPHYCEAE

CONJUGALES

CLOSTERIUM ACEROSUM (Schrank) Ehrenberg. Crater Lake and (doubtful) Mary's Lake; 6,800–8,200 ft.

CLOSTERIUM JENNERI Ralfs, Pl. XLVI, fig. 15. Tank with water-lilies at Ranger Station, Bright Angel Trail, north rim of Grand Canyon; 8,500 ft. (?).

CLOSTERIUM GRACILE DeBrebisson. Walker Lake at 8,300 ft.

CLOSTERIUM LITTORALE Gay? The specimens showed rather too many pyrenoids and were small for this species; they also resemble *C. acerosum minus* Hantsch but were still too small. Wall smooth, no girdle bands, $16\ \mu$ x $133\ \mu$, 7–10 pyrenoids per semi-cell. Deadman's Ranger Station; 6,600 ft.

CLOSTERIUM RALFSII DeBrebisson. Walker Lake at 8,300 ft.

CLOSTERIUM STRIOLATUM Ehrenberg. Tank with water-lilies at Ranger station, Bright Angel trail, north rim of Grand Canyon; 8,500 ft. Apparently differing from the figure and description in West to the extent of showing the striolations as much heavier for a short distance from the cell tips, giving the appearance of a distinct band in this region. Otherwise entirely in agreement.

CLOSTERIUM sps. Undetermined specimens of this genus were occasionally found in the other samples (5 cases) but as solitary or rare examples and not determined.

COSMARIUM DIDYMOPROTUPSUM W. & G. S. West, Pl. XLVI, fig. 14.

Grass Tank and Lake Mary; 5,800–6,800 ft. The determination seems satisfactory; the best alternative is *C. Turpinii podolicum* Gutwinski, which is more sharply truncate-conical, and has smaller lateral protuberances which are closer placed and rather more coarsely punctate.

COSMARIUM FORMULOSUM Hoff, Pl. XLVI, fig. 13. Crater Lake; 8,200 ft. This is probably, according to West, inclusive of *C. mesochondrium* Schmidle, the original figure of which resembles the Arizona plant more closely than does the British one of West (10).

COSMARIUM (near) LAEVE Rabenhorst. Semi-cells rounded triangular-truncate, the ends faintly depressed, membrane smooth, isthmus 0.25 the diameter of the semi-cell, sinus closed, length about $14.5\ \mu$, one pyrenoid in each semi-cell. Grass Tank, upper pool; 5,800 ft.

DESMIDIUM SCHWARTZII Agardh. Tank with water-lilies, Ranger's Station, north rim of Grand Canyon, Bright Angel Trail, abundant; north of camp, V. T. Park; tank 3 miles north of Jacob's Lake; tank 3 miles south of Flagstaff on Lake Mary road; J. P. Dam south of Willows; 6,500–9,000 ft.

MICRASTERIAS RADIOSA Ralfs var. ORNATA Nordstedt, Pl. XLVI, fig. 3. Walker Lake; 8,300 ft.

PLEUROTAENIUM EHRENBERGII (DeBrebisson) DeBary. Walker Lake; 8,300 ft.

STAURASTRUM INCISUM Wolle, Pl. XLVI, fig. 9. Tank with water-lilies, Ranger Station, Bright Angel Trail, north rim of Grand Canyon; 8,500 ft.

STAURASTRUM RADIAN W. & G. S. West. Tank with water-lilies, Ranger Station, Bright Angel trail, north rim of Grand Canyon; 8,500 ft.

STAURASTRUM SEBALDI ORNATUM Nordstedt. Lake Mary; 6,800 ft.

SPIROGYRA WEBERI Kuetzing. Turkey Tanks, fruiting sparingly; 6,000 ft.

SPIROGYRA sps. In addition to the above, 8 samples contained recognizable fragments, or a fair quantity, of sterile *Spirogyra*. Probably several species are represented, for we find: *a*, diameter $21\ \mu$, cells long, end walls plane; *b*, diameter $32\ \mu$, cells 0.5 longer, end wall plane, 1 chromatophore; *c*, diameter $32\ \mu$, cells 11 times longer, end wall plane, 4 chromatophores; *d*, diameter $32\text{--}40\ \mu$; *e*, diameter $35\text{--}40\ \mu$, end wall replicate; *f*, diameter $38\ \mu$, cells long, end wall replicate; *g*, diameter $40\ \mu$, cells long, end wall replicate, 1 chromatophore; *h*, diameter $42\ \mu$, end wall plane, 1 chromatophore; *i*, diameter $50\ \mu$, end wall plane, 1 chromatophore.

VOLVOCALES

EUDORINA ELEGANS Ehrenberg. Mesa, Turkey Hills, and south of railroad; sink north of camp, V. T. Park; Tank, Mund's Park; Lake Mary (dominant 1925); Tank 3 miles south of H. Yeager's Ranch; 5,800–9,000 ft.

GONIUM PECTORALE Muller. South of railroad near Turkey Hills, Lake Mary; 6,800 ft.

PANDORINA MORUM Bory. Mormon Lake; 7,000 ft.

VOLVOX AUREUS Ehrenberg. Dominant in both Lake Mary and tank in Mund's Park, 1925; Walker Lake; 6,400–8,300 ft.

VOLVOX GLOBATOR (Linné) Ehrenberg. South of railroad near Turkey Hills; 6,800 ft.

PROTOCOCCALES

ACTINASTRUM GRACILLIMUM G. M. Smith. Powers Tank, Grass Tank, House Rock Valley; 5,000–6,500 ft.

ANKISTRODESMUS FALCATUS (Corda) Ralfs. Grass Tank; 5,800 ft.

ANKISTRODESMUS FALCATUS MIRABILIS (W. & G. S. West) G. S. West. Chavez Pass, Powers Tank, Grass Tank, Walnut Tank, Oak Creek at Sidiña, House Rock Valley; 4,500–6,500 ft.

CHARACIUM arizonicum W. R. Taylor n. sp., Pl. XLVII, figs. 13–15. Plants cylindrical with rounded apex and obtuse base, strongly curved to nearly straight, stalked, the stalk hardly exceeding 0.33 the diameter of the cell in length, terminated above by a sturdy bristle 0.75–1.5 times the cell diameter in length; cell diameter 10–12.6 μ , length 30–65 μ , rarely to 114 μ , not including the bristle which would add 10–15 μ additional; plants seen had 5–9 chromatophores. This plant differs from *C. gracilipes* Lambert in a generally smaller size and in the rounded, rather than tapering upper end to the cell. It differs from *C. limneticum* Lemmermann in being much more nearly cylindrical and with a proportionately shorter stalk. On crustacea, tank with water-lilies, Ranger Station, Bright Angel trail, north rim of Grand Canyon; 8,500 feet.

CHARACIUM obesum W. R. Taylor n. sp., Pl. XLVII, figs. 22–27. Plants ovoid or subpyriform to elongate oval, symmetrical or somewhat bent upon the longitudinal axis, stalked, the stalk obsolete to half or equal to the cell diameter in length, cells tapering from about the upper third towards the base, the upper third bluntly rounded, without apical thickening or bristle upon the membrane; smaller cells with a single pyrenoid, larger with 2, beyond this the cell contents becoming irregular in arrangement to about 32–64 divisions, probably in relation to internal formation of daughter cells; size without stalk 10.5–13 μ , rarely to 19 μ in diameter, 17–30 μ , rarely to 57 μ long, the larger individuals apparently in reproduction. On crustacea in tank with water lilies, Ranger Station, Bright Angel trail, north rim of Grand Canyon; Jack Smith's Tank; Walker Lake; 7,300–8,500 feet. Differing from *C. Debaryanum* (Reinsch) DeToni, probably the most closely related species, in its somewhat smaller size, more variable and usually shorter stalk, and a generally perceptible asymmetry.

CHARACIUM GRACILIPES Lambert, *fa?*, Pl. XLVII, figs. 16, 17. Plants slender stalked, the stalk nearly one-sixth to one-quarter the length of the individual, expanded somewhat at the attachment end; with a slender bristle at the summit, its length nearly one-sixth to one-quarter the length of the plant, which is nearly straight to slightly curved, tapering evenly from near the middle of the body portion toward both ends, which are acute;

diameter 5-6 μ , length including stalk and bristle 66-85 μ , chromatophores with pyrenoids solitary to about 10 in number, but in these the pyrenoids not clear. The plant seems smaller than the average of Lambert's plants, and was not found attached, so the determination remains somewhat in doubt. It tapers more equally and from more nearly the cell center than does *C. setosum* Filarzsky (6), which has a more acute base and a broader summit, while the terminal swelling or disk on the stalk differentiates it from *C. limneticum* Lemmerman. Jack Smith's tank; 7,300 ft.

COELASTRUM MICROPORUM Naegeli. Grass tank and Walnut Tank; 5,500-5,800 ft.

DICTYOSPHAERIUM PULCHELLUM Wood. Grass Tank; tank with water-lilies, Ranger Station, Bright Angel trail, north rim of Grand Canyon; 5,800-8,500 ft.

DICTYOSPHAERIUM EHRENBERGIANUM Naegeli, var. *minutum* W. R. Taylor, n. var., Pl. XLVII, fig. 7. Cells oval, about 2.0 μ broad by 3.1 μ long, attached to the associating cords by one end, forming groups of fours and apparently multiples thereof, a colony of 16 cells being about 20 μ in diameter, but colonies reaching 128 cells were seen; general gelatine not recognized, the associating cords slender but very well marked. Power's Tank; 6,500 feet.

ELAKATOTHRIX GELATINOSA Wille?, Pl. XLVI, figs. 10-12. Cells 20-35 μ long, 3-4 μ broad, 2-4 in a colony within a wide gelatinous fusiform sheath; cell division transverse, the cells at first with rounded approximated ends, later the opposite side faces elongating so that oblique end faces of the daughter cells are opposed, and finally the mature cells assume a fusiform shape. Differing from the species named in the smaller colonies, and often larger cells, which do not appear to maintain their original position after dividing for any considerable period, in this resembling *E. viridis* (Snow) Printz. Grass Tank; 5,800 feet.

LAGERHEIMIA CITRIFORMIS (Snow) G. M. Smith., Pl. XLVI, figs. 1, 2. Occasional, Walnut Tank; 5,500 feet.

OOCYSTIS BORGEI Snow. Chavez Pass and Walnut Tank; at 5,300-6,300 feet.

OOCYSTIS PARVA W. & G. S. West. Chavez Pass, tank on Walnut Canyon Road, Grass Tank, Walnut Tank; 5,500-6,700 feet. The species of *Oocystis* from the Arizona samples were particularly unconvincing in their imperfect agreement with current descriptions, but it is not often safe to prepare new descriptions in this genus from preserved material. The above two determinations are fairly safe, but in the same samples there were other small forms that did not prove determinable.

PEDIASTRUM BORYANUM (Turpin) Meneghini. Grass Tank, Mud Tank at Roden's Spring; 4,500-5,800 ft.

PEDIASTRUM BORYANUM LONGICORNE Raciborski. Grass Tank, Walnut Tank, more abundant than the above; 5,500-5,800 ft.

PEDIASTRUM DUPLEX Meyen, Pl. XLVI, fig. 16. Vail Lake, very scarce; 7,100 ft.

PEDIASTRUM DUPLEX CLATHRATUM (A. Braun) Lagerheim, Pl. XLVI, fig. 17. Grass Tank, Walnut Tank, occasional; 5,300–5,800 ft.

SCENEDESMUS ABUNDANS (Kirchner) Chodat. Walnut Tank; 5,500 ft.

SCENEDESMUS ABUNDANS LONGICAUDA G. M. Smith. Walnut Tank, Oak Creek at Sidonia; 4,500–5,500 ft.

SCENEDESMUS ARCUATUS Lemmerman. Grass Tank, Walnut Tank; 5,500–5,800 ft.

SCENEDESMUS BIJUGA ALTERNANS (Reinsch) Borge. Walnut Tank; 5,500 ft.

SCENEDESMUS DENTICULATUS Lagerheim. Walnut Tank, Lockett's Gardens; 5,500–7,200 ft.

SCENEDESMUS DIMORPHUS (Turpin) Kuetzing. Iron Tank at Roden's Spring, House Rock Valley; 4,500–5,000 ft.

SCENEDESMUS OBLIQUUS (Turpin) Kuetzing. Iron Tank at Roden's Spring, Oak Creek above Lodge; 4,500–5,900 ft.

SCENEDESMUS QUADRICAUDA (Turpin) Brebisson. Walnut Tank; 5,500 ft.

SCENEDESMUS QUADRICAUDA PARVUS G. M. Smith. Grass Tank; Walnut Tank; 5,500–5,800 ft.

SCENEDESMUS QUADRICAUDA QUADRISPINA (Chodat) G. M. Smith. Grass Tank; 5,800 ft.

SCHROEDERIA JUDAYI G. M. Smith, Pl. XLVII, fig. 12. Crater Lake, Grass Tank; 5,800–8,200 ft.

SELENASTRUM MINUTUM (Naegeli) F. S. Collins, Pl. XLVII, figs. 1–6. Power's Tank, Grass Tank, Iron Tank at Roden's Spring, Walnut Tank; 4,500–6,000 ft.

TETRAEDRON CAUDATUM (Corda) Hansgirg, Pl. XLVI, figs. 7, 8. Grass Tank, Walnut Tank; 5,500–5,800 ft.

TETRAEDRON MINIMUM (A. Braun) Hansgirg, Pl. XLVI, fig. 5. Grass Tank; 5,800 ft.

TETRAEDRON REGULARE INCUS Teiling, Pl. XLVI, fig. 6. Grass Tank, Walnut Tank; 5,500–5,800 ft.

TETRAEDRON REGULARE LONGISPINUM Reinsch fa?, Pl. XLVI, fig. 4. Cell small, without spines the arms spreading 16μ , with spines 32μ , tetrahedral, the sides compressed and the isthmus about 5μ across. Differing from the variety named in being about half the ordinary size. Rare, Power's Tank; 6,500 ft.

ULOTRICHALES

CHAETOSPHAERIDIUM GLOBOSUM (Nordstedt) Klebs. Scarce, Oak Creek above Lodge; 5,900 ft.

OEDOGONIALES

OEDOGONIUM sp. Filament above about $11.5\ \mu$ in diameter, towards the summit tapering into an extremely long and slender hair, below near the base cells about $7.5\ \mu$ in diameter, throughout about 4 times as long as broad, the membrane smooth; spores scattered, the oögonia round-oval to ovoid, $29\text{--}30\ \mu$ in diameter and $32\text{--}44\ \mu$ long, probably with circumcissile dehiscence. As the male plants were not seen and as the spores were not mature it is hardly possible to give an exact name to this species. Grass Tank; 5,800 ft.

HETEROKONTAE

BOTRYOCOCCUS BRAUNII Kuetzing. Mary's Lake, Marshall Lake; 6,800–7,100 ft.

OPHIOCYTIUM COCHLEARE A. Braun, var. *inflatum* W. R. Taylor n. var., Pl. XLVII, figs. 8–11. Cells $12\text{--}16\ \mu$ in diameter, $200\text{--}320\ \mu$ long, the wall thick, to $3.8\ \mu$, straight or moderately curved, the base somewhat inflated, the apex crowned by a spine $15\text{--}20\ \mu$ long. Differing from *O. minus* Naegeli in that the spine is not knobbed at the end, and in being rather smaller, if the measurements given by Pascher (Süßwasserflora Deutschlands u.s.w., Heft 11), are to be accepted rather than those of DeToni (Sylloge Algarum). Differing from *O. cochleare* A. Braun in being larger, and not so markedly twisted. A few specimens were seen with a transverse wall dividing the individual into 2 cells, but one was empty in each case. The membrane at the aperture in those empty cells which were found with the spine-tipped cap missing was markedly obliquely lamellose. Walker Lake; 8,300 ft.

OPHIOCYTIUM PARVULUM (Perty) A. Braun. Grass Tank; 5,800 ft.

POSITION UNCERTAIN

AMOEBIIDIUM PARASITICUM Cienkowski, var. *Coltoni* W. R. Taylor n. var., Pl. XLVII, figs. 18–21. Plants straight to moderately curved, sessile, the membrane considerably thickened and a little expanded at the base as a holdfast, tapering gradually and approximately equally from the middle toward both ends, the apex rounded and unarmed, the membrane here not appreciably thickened; cell diameter $6\text{--}15\ \mu$, generally about $11\ \mu$, length $35\text{--}80\ \mu$, generally about $50\ \mu$, nuclei 4–10, divisions between these generally indistinct; reproduction involving internal formation of about 32 fusiform to lunate cells. On crustacea, Roden's Spring Mud Tank, tank 3 miles south of Flagstaff by Mary's Lake road, Jack Smith's tank, and Deadman's Ranger Station; 4,500–7,300 ft. Attention was first called to this plant in the Mud Tank material by Dr. Colton, though it proved to be much more abundant in the other samples, especially that from Jack Smith's tank. In preserved material, associated with species of *Characium* and *Characiopsis* which have become decolorized, it is easy to mistake vegetative individuals of this plant for members of these two algal genera. The finding of abundant

material of what appears to be the original species in cultures of *Daphnia* at the University of Pennsylvania enabled the writer to follow out most of the life history, and to make inquiries which resulted in most helpful suggestions and a tentative determination of the Pennsylvania material by Professor W. C. Coker, of which acknowledgment is gratefully made. In decolorized preserved samples the lunate cells readily distinguish this Arizona *Amoebidium* from *C. cylindricum* Lambert (7), *C. saccatum* Filarszky (6, 9), and *Characiopsis groenlandicum* (P. Richter) Lemmerman or its var. *rossica* Elenkin (4, 5), while *C. saccata* Carter (1) has a short stalk and is sigmoid rather than lunate in habit when asymmetrical. There is also some resemblance to the genus *Harpochytrium*, but the method of spore formation given in the illustrations will sufficiently indicate the relation to *Amoebidium* as described by Cienkowski (Bot. Zeit. 19: 169-174, pl. 7, 1861). The genus has been variously interpreted as of fungal or of animal affinities, but is included here because of its interesting character and to attract attention to its presence in the Arizona plankton. The distinction from the original species is made on the basis of shape differences, for *A. parasiticum* is lunate in its youngest stages only, becoming cylindrical as it matures, and is seldom over 10 μ in diameter, while the Arizona plant averages 11 μ .

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DESCRIPTION OF PLATES

PLATE XLVI

FIGS. 1, 2. *Lagerheimia citriformis*, two specimens showing 2 and 4 young daughter cells, $\times 1200$, $\times 1500$.

FIG. 3. *Micrasterias radiata ornata*.

FIG. 4. *Tetraedron regulare longispinum?* $\times 770$.

FIG. 5. *Tetraedron minimum*, $\times 770$.

FIG. 6. *Tetraedron regulare incus*, $\times 770$.

FIGS. 7, 8. *Tetraedron caudatum*, $\times 770$.

FIG. 9. *Staurostrum incisum*, polar view, $\times 1025$.

FIGS. 10-12. *Elakatothrix gelatinosa?* Three colonies, showing most striking features of cell division, $\times 920$.

FIG. 13. *Cosmarium formulosum*, lateral view, the flattening of the polar areas being more obvious in the living cell than in the drawing, $\times 680$.

FIG. 14. *Cosmarium didymoprotupsum*, lateral view, the lateral protuberances being shown only in outline, $\times 625$.

FIG. 15. *Closterium Jenneri*, $\times 750$.

FIG. 16. *Pediastrum duplex*, $\times 560$.

FIG. 17. *Pediastrum duplex clathratum*, $\times 1425$.

PLATE XLVII

FIGS. 1-6. *Selenastrum minutum*, $\times 1535$.

FIG. 7. *Dictyosphaerium Ehrenbergianum minutum*, $\times 1158$.

FIGS. 8, 9. *Ophiocytium cochleare inflatum*, individuals, $\times 225$.

FIGS. 10, 11. *Ophiocytium cochleare inflatum*, apex and base of cells, $\times 920$.

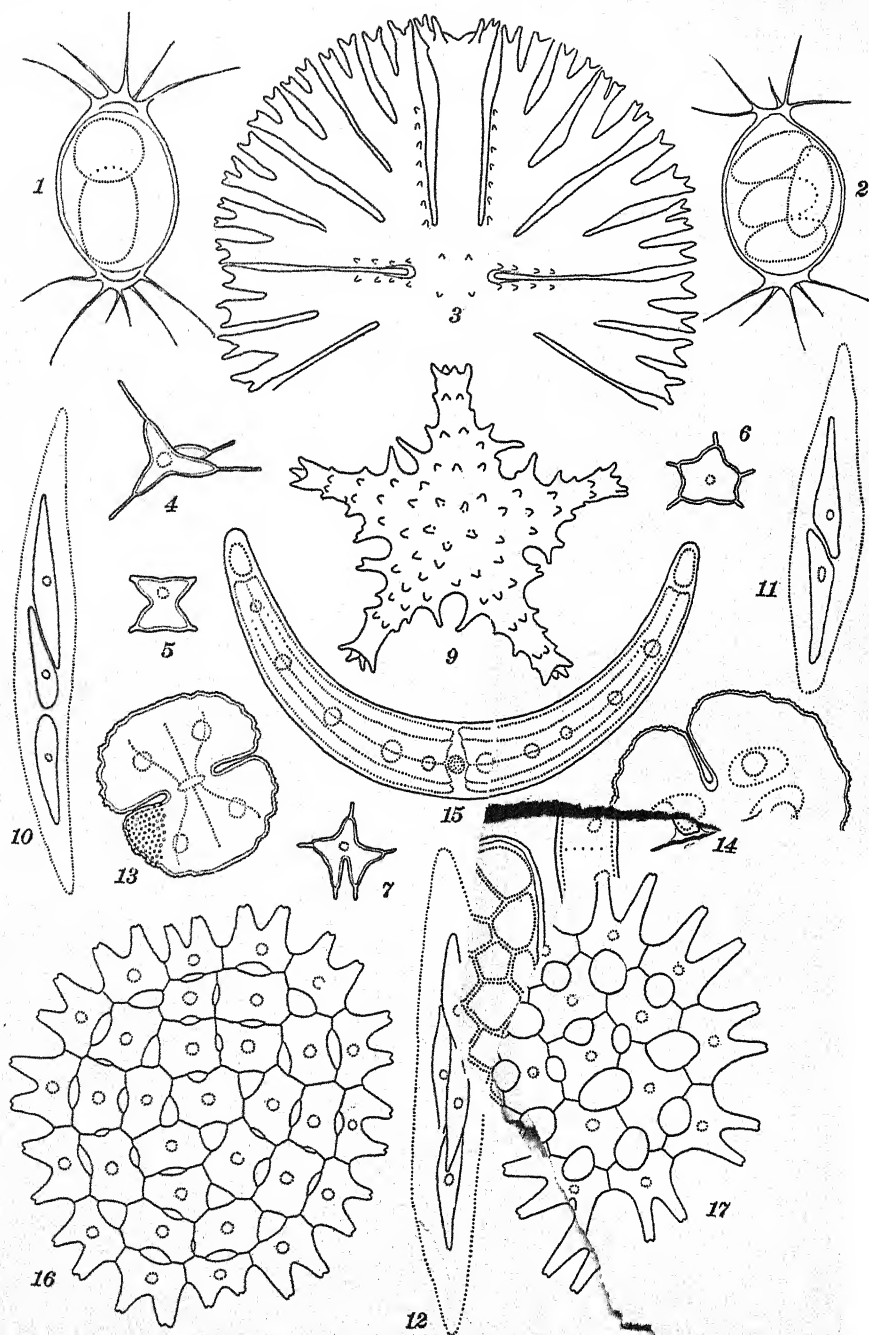
FIG. 12. *Schroederia Judayi*, $\times 770$.

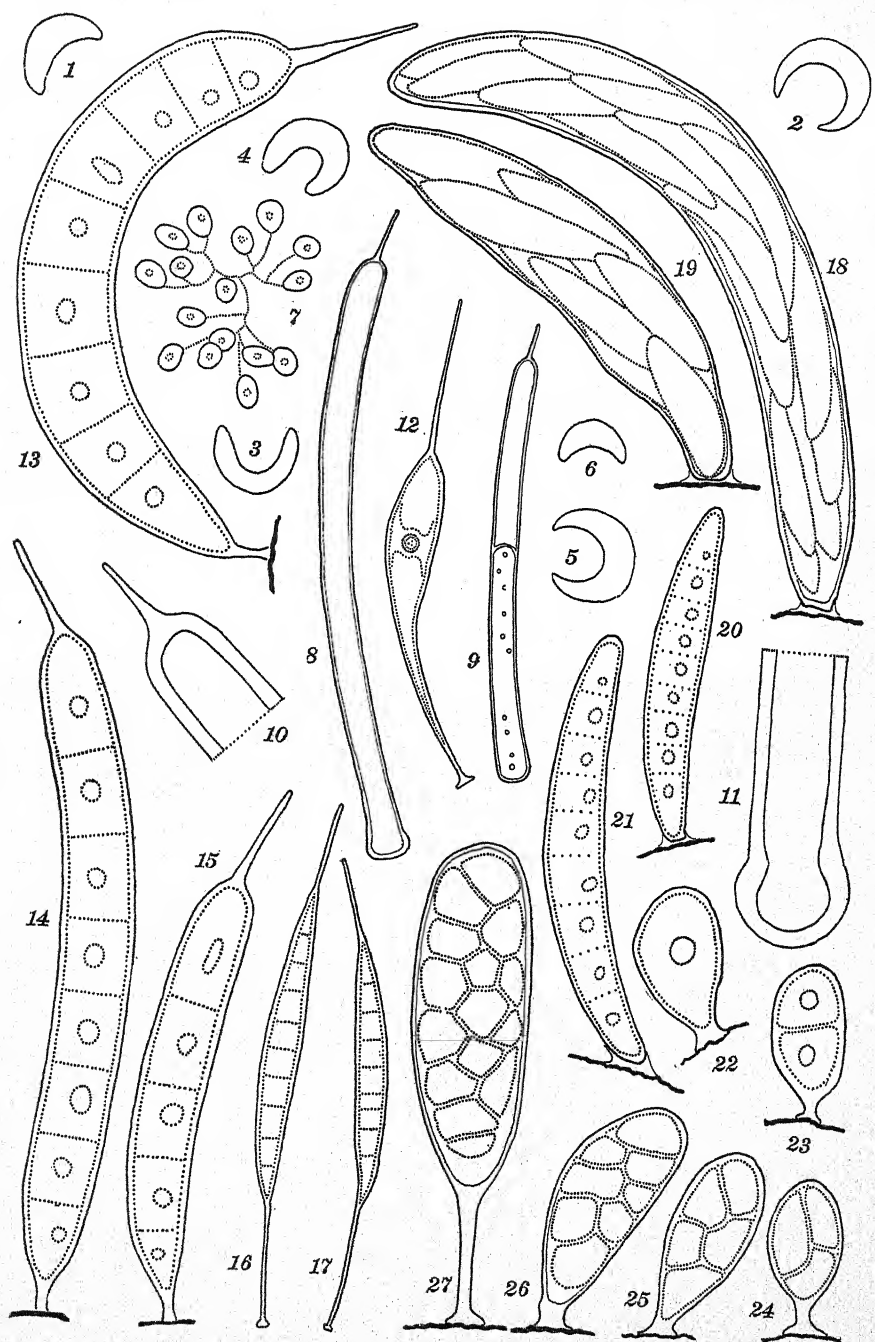
FIGS. 13-15. *Characium arizonicum*, three individuals, $\times 1200$, $\times 920$, $\times 1200$.

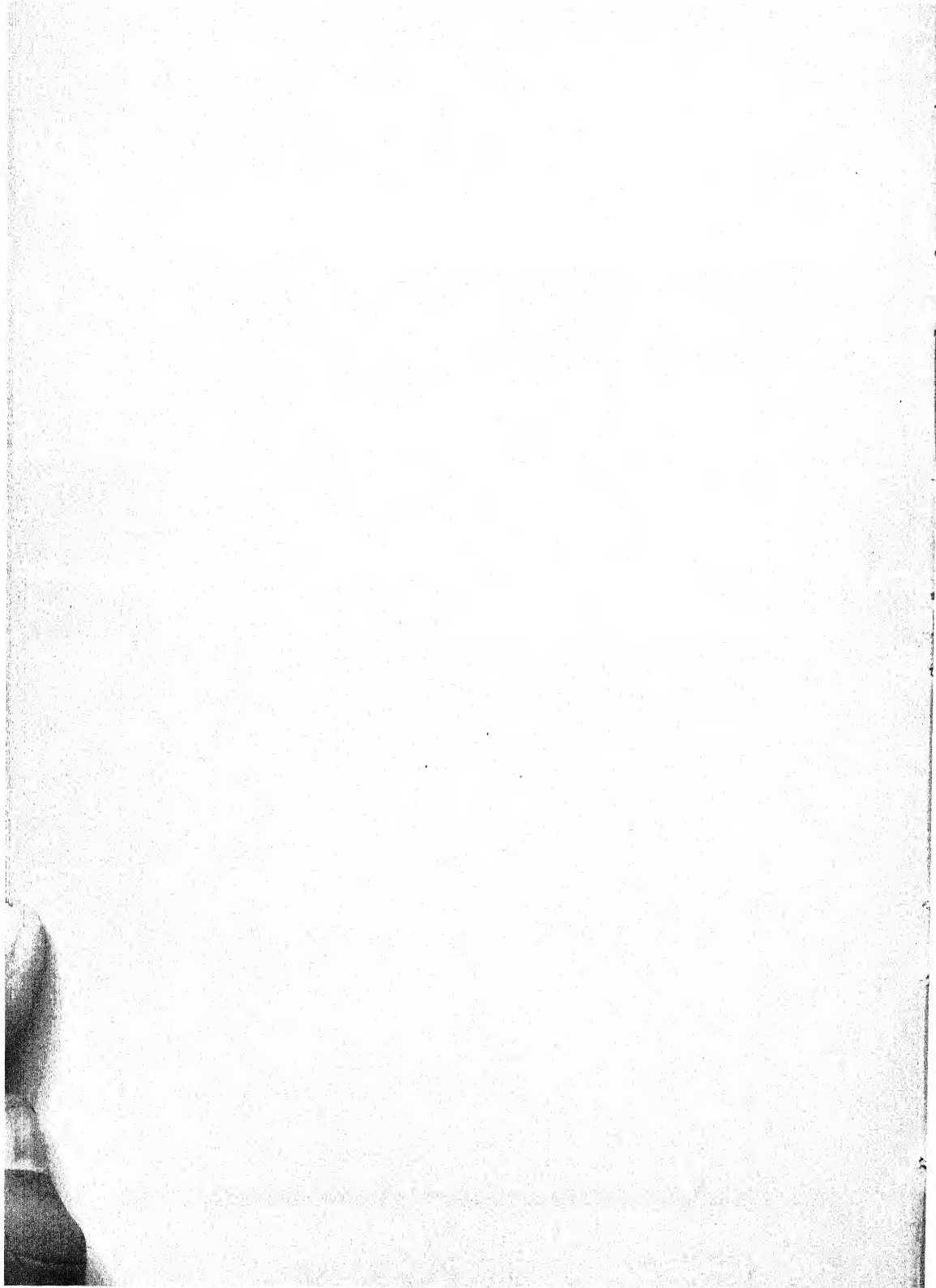
FIGS. 16, 17. *Characium gracilipes* fa? two individuals, $\times 920$.

FIGS. 18-21. *Amoebidium parasiticum* Coltoni, reproducing, $\times 1200$, $\times 1535$; and vegetative individuals, $\times 1055$.

FIGS. 22-27. *Characium obesum*, vegetative and reproducing individuals, $\times 920$.







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ABSTRACTS OF THE PAPERS PRESENTED BEFORE THE PHYSIOLOGICAL SECTION OF THE BOTANICAL SOCIETY OF AMERICA, NEW YORK, N. Y., DECEMBER 27-29, 1928

A Microchemical Study of the Structure of an Epidermal Cell Wall.

Donald B. Anderson, North Carolina State College, Raleigh, N. C.—The researches of A. Frey upon the optical properties of the outer epidermal cell wall of *Clivia nobilis* have shown: (1) a conspicuous isotropic zone in the center of the wall; (2) zones on either side of this isotropic region with a maximum double refraction at their centers and not at their margins. This paper suggests an explanation of these interesting facts from a microchemical point of view. A microchemical study of this cell wall shows it to be composed of cutin, cellulose, and pectic compounds. These membrane substances are associated in the cell wall in the following manner: (1) an outer layer of pure cutin; (2) a zone of cutinized cellulose; (3) a zone of cutinized cellulose containing pectic compounds; (4) a zone of pectic material; (5) a zone of cellulose and pectic materials adjacent to the cell lumen. The relations existing between these compounds explain satisfactorily the peculiar optical properties of this cell wall.

Concentration of Iron-ions and Growth in Culture Solutions. E. F.

Hopkins, Cornell University, Ithaca, N. Y.—It has been found, with relatively large amounts of iron present in true solution in a nutrient medium, that this iron may not be available for plant growth. By increasing the sodium citrate content of the culture solution it has been possible to decrease and finally completely to inhibit the growth of *Chlorella* sp., which effect may be brought about at practically any iron concentration if sufficient citrate is used. The phenomenon can be logically explained on the basis of complex ion formation. If we add sodium citrate to a solution containing a ferric salt there results the formation of the complex, sodium iron-citrate, in which the iron is present in the anion and no longer gives the usual reactions for ferric iron. This complex, which contains two atoms of iron to three citrate residues, ionizes principally into Na-ions and iron-citrate-ions. The complex ions, although very stable, ionize themselves to an appreciable extent, furnishing a minute amount of iron-ions which, though small, is sufficient to give good growth of *Chlorella*. However, with further increase in the citrate content the iron-ion is depressed

until finally there is insufficient iron available for growth. In the same manner we may account for decreased toxicity of iron and also for the solubility of iron in alkaline solution when citrates are present. It is concluded from these facts that iron is available for growth only in the form of its ions and, therefore, that the amount of growth is dependent on the iron-ion concentration. Experimental studies have shown that in such solutions as those above described both ferric and ferrous ions are present, and that their concentration may be changed by changing the amount of citrate-ion.

Studies on the Growth of Root Hairs in Solutions—XIII. The pH-Molar Rate Relation for Collards in Calcium Sulfate. *Wanda K. Farr, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—Studies upon the development of root hairs of *Brassica oleracea* have been made in solutions of calcium sulfate according to methods previously used by C. H. Farr and the author in solutions of calcium hydroxid, calcium chlorid, and calcium nitrate. The values obtained in 0.000448 *M* CaSO_4 were apparently functions of the $\text{Ca}(\text{OH})_2$ added to increase the alkalinity of the solution. A salt concentration of 0.000540 *M* produced an effect upon the rate of growth which apparently supplemented the effect of $\text{Ca}(\text{OH})_2$ between pH 8.5 and pH 10. The first typical trimodal calcium sulfate curve was produced in solutions of 0.000714 *M* concentration. In this and in all higher salt concentrations there were two distinct alkaline maxima and one acid maximum showing close correspondence, in the different curves, in their relative positions upon the pH scale. The results in calcium sulfate are notable for the high rates of growth in solutions of low molar concentration. Morphological changes in root hairs in response to the various solutions used were studied and compared with those in solutions of other calcium salts. While some effects may be peculiar to the sulfate solutions, the evidence indicates that the most important causal agencies in the production of these abnormalities are the hydrogen- and hydroxyl-ion concentrations of the culture solutions.

Experiments on Boron Tolerance of Citrus Plants and their Wild Relatives. *Walter T. Swingle, T. Ralph Robinson, and Eugene May, Jr., U. S. Dept. Agr., Washington, D. C.*—The boron tolerance of citrus plants and related plants belonging to other genera was tested by watering a number of plants growing in pots in the greenhouse with solutions containing various proportions of dissolved boron salts. The first experiment was begun in June, 1927, watering two comparable batches of plants, one with water containing one part per million of boron in solution, the other with water containing 10 parts per million of boron. The latter group of plants soon showed distress, and the lot receiving even one part per million began to show more or less distress within 2 or 3 months. Sour

orange, bitter sweet orange, Cleopatra mandarin, and calamondin showed slight resistance. Outstanding boron tolerance was shown by *Severina buxifolia* (the Chinese box orange), *Eremocitrus glauca* (the Australian desert lime), and *Atalantia disticha* (a Philippine species), but all three were injured by 10 parts per million. Many repetitions of the experiments since June, 1927 have given concordant results. The boron content of the soil steadily increased with continued watering, and experiments using nutrient solutions to which small amounts of boron have been added are now under way. The species mentioned above showing boron tolerance can be used as root-stocks for lemons, grapefruit, oranges, and other citrus fruits. This affords a good example of the possibility of utilizing as stocks the wild relatives of cultivated plants for extending their cultivation into regions where such wild stocks are better suited to the soil.

Further Studies as to the Effect of Mineral Nutrients upon Seed Plants. Phosphates. *Thomas W. Turner, Hampton Institute, Hampton, Va.*—A study was made of the mechanism of the effects of phosphates upon the growth of roots by means of water-culture experiments, using barley, wheat, and cotton. Increasing the phosphate concentration decreased the ratio of tops to roots. Nitrates were shown in previous studies to give the opposite results. Whether or not the effect of phosphates in bringing about relatively greater root growth as compared with tops resulted from a directly stimulating action of these salts upon underground portions was tested by employing Robbins' method of growing root tips under pure-culture conditions. While the same solutions were used as in the water cultures above, root tips only of corn were dealt with. These pure-culture experiments are reported in three series, and are strikingly constant in showing that increasing the phosphate concentration not only does not have the effect of stimulating directly growth in length or multiplication of lateral roots, but both of these are retarded under such conditions. The experiment shows that cellular activity which should manifest itself in increased growth in length or in multiplication of secondary roots is not increased by direct application of phosphates as is implied in the usual statements. The actual facts noted, then, that there is a decreasing ratio of tops to roots as the phosphate concentration is increased, must find explanation in the formation of compounds or simple substances in connection with photosynthetic activity in the tops, which are translocated to the roots and manifest themselves there by their stimulating or storage effects.

On the Penetration of Methylene Blue into Living Cells. *Matilda Moldenhauer Brooks, University of California, Berkeley, Calif.*—The studies on the penetration of methylene blue into living cells have been continued, using the fresh-water alga *Nitella* and the larger species of the marine alga

Valonia (*V. ventricosa*). The spectrophotometric analyses of the sap of these plants which had been placed in buffered solutions of methylene blue at pH's 5.8 and 9.0 for about 3 hours, showed that the primary absorption maximum was about $660\ \mu\mu$, while the secondary maximum was at 600 to $610\ \mu\mu$. Inasmuch as these wave-lengths are the absorption maxima of methylene blue itself and not of any of its lower homologs, the conclusion is drawn that methylene blue as such penetrates the living cells. These results are in agreement with the writer's previous findings on the penetration of methylene blue into *Valonia macrophysa*. (Brooks, M. M. University of California Publications 31: 79. 1927. Proc. Nat. Acad. Sci. 13: 821. 1927.)

Electrometric Determination of Initial and Total Acidity of Juices Extracted from the Leaves of Green and Variegated Varieties of *Evonymus japonicus*. Everett F. Davis, *Virginia Agr. Exp. Sta., Blacksburg, Va.*—A modification of the usual quinhydrone electrode method made possible pH determinations with a single drop of plant juice. About 400 determinations of the acidity of juice from green leaves of *E. japonicus*, and from the chlorotic and green areas of the variegated types, "argenteo," "mediopicta," and "aurea," were made. Comparisons of acidities of chlorotic and green areas, of young and old leaves, of frozen and sound tissues were made. Exposing the extracted juice to air caused an increase in the initial acidity to an extent roughly proportional to the period of exposure. Titration curves (using 1 to 2 cc. of extracted juice) showed relatively little buffer action of juice from leaves of variegated varieties as compared with the green variety. With *E. japonicus* var. "mediopicta," there is some indication that the chlorotic-area juice had lower buffer capacity than juice from the green areas or from entire variegated leaves. Juice which was expressed from frozen leaves showed a pronounced increase in buffer action over that expressed from similar but unfrozen leaves.

A Comparison of the Glass Electrode with Other Methods for the Determination of pH on Physiological Materials. W. J. Youden and I. D. Dobrosky, *Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—Acidity determinations may be made on very small samples, and without the loss of the sample, by means of the glass electrode. This method depends on the establishment of an e.m.f. between two fluids when separated by a very thin glass membrane. The magnitude of the e.m.f. is proportional to the difference in the pH of the fluids; its measurement, however, is complicated by the exceedingly high resistance of the system. A compact arrangement for this purpose has been constructed which differs by several simplifications from those heretofore described. The apparatus has been tested on an extensive variety of materials, such as the juices of fruits,

vegetables, tubers, leaves, stems, roots, fungi, and animal tissue. Comparative measurements of the pH of the same specimens were made with the hydrogen electrode, the quinhydrone electrode, and the colorimetric method. The glass electrode promises to become an important device for the measurement of acidity by virtue of the ease and rapidity of operation and especially through the avoidance of any alteration of the sample. (Demonstration of the glass electrode will be made at the section meeting.)

The Effect of Various Animal Anesthetics upon the Seismic Movements of *Mimosa pudica*. *Raymond H. Wallace, Columbia University, New York, N. Y.*—Careful quantitative experiments made from April to September have shown that ether is the only common animal anesthetic which will actually suspend the seismic movements of *Mimosa pudica*. Concentrations of ether vapor from 13 to 25 percent (to volume of air) will prevent all movements of leaflets and petioles within from ten to thirty minutes after exposure. Concentrations of chloroform of 2.5 percent or greater are lethal, while concentrations lower than these may cause injury. The lethal concentration (2.5 percent) of chloroform reduces the amount of movement of the primary petiole less than 40 percent. *Mimosa* plants will retain their sensitivity from 1 to 4 hours in 100-percent nitrous oxid or ethylene. Repeated anesthetizations by ether, or repeated exposures to nitrous oxid or ethylene, have little or no harmful effect on the plants. Curves showing the relations of various concentrations of ether, chloroform, carbon tetrachlorid, carbon disulphid, nitrous oxid, methyl alcohol, and ethyl alcohol, on the movement in degrees of the primary petioles are given.

The Effect of Acetylene on the Ripening of Bananas. *R. Hartshorn, Cornell University, Ithaca, N. Y.*—Placing fruit in containers with carbide or passing moist air over carbide and then through respiratory chambers increased the rate of ripening of bananas as indicated by changes in color, pressure, flavor, starch content, and respiratory rate. The effect was marked with chilled fruit. The amount of carbide could be varied within wide limits with the same results.

Extracts of Flax Toxic to Fungi. *Ernest S. Reynolds, Washington University, St. Louis, Mo.*—Numerous quantitative determinations of the hydrocyanic acid content of flax by a modified Roe aeration method have been made. This biochemical product, which is highly toxic to certain fungi, especially to *Fusarium lini*, is exceedingly variable in quantity, depending upon the age and variety of flax and upon the complex of environmental conditions during the growth of the flax. Dilute water extracts of flax stimulate the growth of *F. lini* in culture, while extracts approximating the natural concentration of flax juice entirely inhibit the develop-

ment of this fungus. The water-soluble portions of the ether extracts of certain strains of flax are also very toxic to the fungus. The toxic material of flax has been extracted by various solvents and within certain limits the rate of growth of the *Fusarium* is directly proportional to the degree of dilution of this injurious extract.

The Toxic Principle of *Juglans nigra* as Identified with Synthetic Juglone, and its Toxic Effects on Tomato and Alfalfa Plants. *Everett F. Davis, Virginia Agr. Exp. Sta., Blacksburg, Va.*—Attention has been called, by workers at the Virginia Agricultural Experiment Station, to the toxic effects of mature black walnut (*Juglans nigra*) trees toward certain plant associations grown near them. Severe cases of similar injuries in apple orchards in Virginia have resulted in the death of apple trees which have been described. Following these observations, the present author, at the Virginia Agricultural Experiment Station, has sought to identify the toxic principle found in various parts of the walnut tree with juglone, which is known to occur throughout all parts of the plant. A substance has been extracted and purified, by a method for extracting juglone, from the hulls and roots of *Juglans nigra*. The purified and crystalline extract, in each case, has proved to be an exceedingly powerful toxin when injected into the stems of tomato and alfalfa plants. Juglone, or the 5-hydroxy-alpha-naphtha-quinone, has been synthesized by oxidation of alpha-naphtha-quinone. A comparison of its melting point with that of the toxic extract from *Juglans nigra* has shown these substances to be identical. Similar toxicity has been exhibited by both the synthetic and natural products.

The Second Cycle of the Growth of a Population of Yeast. *Oscar W. Richards, Clark University, Worcester, Mass.*—A population of yeast reaches an equilibrium number about 100 hours after planting a small seeding of yeast in a synthetic culture medium at 28° C. The equilibrium is due to a selective destruction of the larger buds by the toxic excretion products of the cells, while the larger resistant cells continue to form buds. The fragmentation of the injured cells results in an increase of the carbohydrate content of the medium. The second cycle of the growth of the population begins in about 130 hours and continues until a second equilibrium number is attained about 200 hours after seeding the medium. The increase in the number of cells present is associated with the increase in the carbohydrate content of the medium and a decrease in the amount of alcohol present. The acidity (pH) of the culture fluid remains practically constant from 100 to 200 hours after seeding and may decrease slightly from 200 to 300 hours. The percentage of injured cells, determined by their becoming stained with methylene blue, increases from 40 hours after seeding until during the second growth cycle when the percentage remains nearly constant, after which the percentage increases steadily to the end

of the period of observation. The analysis of the relation between these observations and the change in the size distribution of the cells present during the second growth cycle will be used to indicate the conditions which determine the second cycle of growth of a population of yeast.

Effect of Polarized Light on Plants. *David I. Macht, Hynson, Westcott, and Dunning, Baltimore, Md.*—The effect of polarized light was studied on growth and germination of *Lupinus*, wheat, *Digitalis*, sunflower, and other plants, and also on the hydrolysis of starch and ripening of fruit. Two classical methods of obtaining polarized light were employed: In one method a Nicol Prism was utilized while in the other polarization was secured by piles of glass plates placed at the polarizing angle in respect to the source of light. The wave-lengths studied were those belonging to the visible spectrum, and also to some extent the short infra-red wave-lengths, but not those of the ultra-violet region. Controls were made with non-polarized light of exactly the same intensity and quality. Numerous experiments showed that polarized light stimulated the growth of various seedlings and especially of *Lupinus albus* with which the majority of experiments were carried on. A hastening of germination was also noted in many of the experiments. Polarized light was found to hasten the conversion of starch into sugar in the presence of diastase. Ripening of tomatoes was much hastened by exposure to polarized light as compared with simultaneous control experiments on other specimens exposed to non-polarized light from the same source. A method for securing polarized light for practical purposes is to use transparent or translucent cellulose films. The most convenient and practical way of doing this is by the use of celloglass. Experiments with light passing through celloglass give qualitatively the same results as those obtained with polarized light produced by classical physical methods. The historical significance of the above experiments is also of interest.

The Influence of Light Intensity and Quality upon the Growth of Plants. *Hardy L. Shirley, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—Plants were grown under a series of shades located both in the greenhouse and outside, each provided with forced ventilation. The curve of increase in dry weight with increasing light intensity is almost a straight line for sunflowers and *Galinsoga* during the winter. During the summer the slope of the curve falls off at higher intensities, there being little increase in dry weight at intensities above 50 percent of full sunlight. With *Geum* and buckwheat the slope decreases at lower intensities compared to sunflower and *Galinsoga*. Slight shading in summer seemed beneficial for many plants. Experiments with plants grown under artificial light gave similar results. Sunflowers, *Geum*, and *Galinsoga* were grown under five different light qualities with three intensities in each quality. The qualities were

secured by filtering sunlight through Corning glasses having the following transmission ranges: wave-lengths 290-720, 374-585, 389-720, 472-720, and 529-720 millimicrons. The plants studied produced more dry matter per unit light intensity under the complete solar spectrum, quality 1, than under any portion of it. Plants grow more efficiently without the red, quality 2, than without the blue, quality 5. The relative efficiency of the different qualities for the production of dry matter is as ordered above.

The Chlorophyll Content of Normal and Mosaic Leaves of Tobacco. A. A. Dunlap (introduced by C. G. Deuber), Yale University, New Haven, Conn.—Quantitative determinations of the chlorophyll (a + b) of mosaic and healthy tobacco plants were made throughout the growing period. Comparisons of these determinations show a reduction of the green pigments in the mosaic leaves at all stages of growth. The reduction in chlorophyll content was found to be greater in the young and also in the oldest mosaic leaves of these plants.

Genetic and Other Effects of X-ray and Radium Treatment of Seeds, Growing Points, and Sex Cells of *Nicotiana* Species. T. H. Goodspeed and A. R. Olson, University of California, Berkeley, Calif.—Five species of *Nicotiana*—*Langsdorffii*, *syvestris*, *glutinosa*, *rustica*, and *tabacum*—are being employed in experiments designed to give a general survey of the reaction of plant tissues of various kinds to X-rays and radium. The information being obtained is being utilized in genetic, cytological, and physiological investigations dealing with (1) the relation of dosage and stage of maturity to incidence of variation and the production of certain types of histological and cytological modification and (2) the effects of irradiation on growth and development, in particular its relation to viability of seeds and pollen and to the incidence of bud abscission. Certain preliminary conclusions may be drawn from the evidence already obtained: (1) in all the species investigated the immediate progenies from treated somatic or reproductive tissues exhibit marked evidence of variation; (2) a certain amount of this variation is a consequence of disturbances of mitotic or meiotic elements or mechanisms the results of which are visible, possibly cytoplasmic in origin and directly referable to the particular character of the treatment; (3) few purely somatic variations occur; (4) all investigated variant characters which appeared in the immediate progeny of X-rayed sex cells are inherited and a number of recessive modifications have occurred; (5) a marked acceleration of growth and development may follow appropriate X-ray treatment; (6) the curve of resistance to X-rays and radium differs significantly in the case of seeds, growing points, maturing sex cells, and mature pollen; (7) the extent of flower-bud abscission is intimately related to X-ray dosage.

Effect upon *Digitalis purpurea* of Radiation through Solarized Ultra-violet-transmitting Glass. *Adelia McCrea, Dept. of Medical Research, Parke, Davis & Co., Detroit, Mich.*—In a series of tests upon a group of plants of *D. purpurea*, the results obtained last year were confirmed. Plants were exposed during the seedling stage under the ultra-violet-transmitting glass which, in the greenhouse roof, had been solarized about thirteen months. After later culture under garden conditions, the radiated plants proved more potent than the controls by 21.62 percent to 40 percent.

Killing of Plant Tissue and Mosaic Virus as Related to Wave-length in the Ultra-violet Region. *John M. Arthur, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—The purpose of the first part of this investigation has been to determine what region of the ultra-violet component of the radiation from a mercury vapor arc in quartz is most injurious to plants, and further, whether that region near wave-length $290\ \mu\mu$, the extreme limit for solar radiation, is injurious. Using appropriate filters it has been found that the injury begins at a wave-length shorter than $285\ \mu\mu$ and increases rapidly with decreasing wave-length. The second part of the investigation was a determination of the time necessary to get complete killing of the virus which causes tobacco mosaic when suitably exposed, first directly to the radiation from a mercury vapor arc in quartz and second through filters. It was found that the killing time of direct exposure is less than 15 seconds.

Is Ultra-violet Radiation Stimulating to Plants? *H. W. Popp and Florence Brown, Pennsylvania State College, State College, Pa.*—The present paper reports the results of experiments in which germinating seeds of turnip, cucumber, *Amaranthus*, *Rumex*, and other species were subjected to various dosages of ultra-violet radiation as obtained from a Cooper-Hewitt Mercury Vapor Lamp in quartz. In some cases the full spectrum from this lamp was used; in others only the region between 400 and 300 millimicrons. Controls were kept in darkness, in diffused light, and in light from which all ultra-violet radiation was eliminated; other controls were exposed to the ozone from the lamp without being subjected to its radiation. The results to date indicate that daily exposures of five seconds or more to the full spectrum from the lamp, operated at a distance of 50 cm. from the plants, result in deleterious effects on all seedlings investigated. In no case was stimulation obtained. Similar exposures in the region between 400 and 300 millimicrons were neither stimulating nor markedly inimical to the growth of seedlings that were kept in diffused light or in light from which all ultra-violet was screened off. In general, seedlings kept in the dark were unaffected by this region of the spectrum when the daily exposures were one minute or more. A doubtful case of greater elongation of the hypocotyls of turnip seedlings kept in the dark was obtained in one series

in which the daily exposures were five to sixty seconds. The experimental conditions and results of this investigation are compared with those of other investigators.

Further Experiments on the Growth of Plants in a Controlled Environment—I. The Relation of Light Intensity and Exposure Time to Yield.

II. The Interrelationship of Temperature and Light. *A. R. Davis and D. R. Hoagland, University of California, Berkeley, Calif.*—I. One hundred

seedlings of a pure line strain of Little Club wheat were grown under controlled light, temperature, and culture-solution conditions for four-week periods. Illumination was supplied by 300 watt, Mazda C, gas-filled lamps and in the several experiments was varied with respect to intensity and daily exposure time, the former ranging between 1200 and 3000 c.p., and the latter between 4 and 24 hours. In all cases the temperature was held at 20.5° C. and the probable error of the mean dry weight was approximately 1 percent. The $IS = k$ relationship (I = intensity, S = daily exposure period, k = yield constant), as suggested by Gregory and others, was found not to hold under our conditions. Yield proved to be an exponential function of daily exposure time where intensity was constant, between the limits 4 and 12 hours, while between 12 and 22 hours the function became that of a straight line with a slope of 1.48. With intensity the variable and exposure time constant yield was again a straight line function but with a slope of 0.87. The suggestion is that the plant may function more efficiently from the point of view of tissue production where the radiant energy available is distributed over a longer period of time at a lower intensity than when the opposite condition holds. II. Where illumination was held constant and temperature varied between 14° and 30° C. the curve for yield reached a maximum at 25° C. and then fell off sharply, indicating a critical point between the rate of respiration and that of photosynthesis. This suggests that for any given illumination value a critical temperature exists which for a given plant should be possible of prediction. The same type of relationship between the total amount of energy absorbed and that released through oxidation is shown by the steady increase in the top-root ratio with increasing temperature. Presumably, since light is the limiting factor, there is less excess carbohydrate material at the higher temperatures, and the roots, because of their location, are the first to be restricted in the use of such excess for tissue formation.

Is the Fungus Necessary for Development of Seedlings of *Calluna vulgaris*? *Lewis Knudson, Cornell University, Ithaca, N. Y.*—The work of

Raynor on the distribution of the fungus in *Calluna vulgaris* and experiments made by her to demonstrate the necessity of the fungus for proper development of the seedlings are well known. Christoph reported satisfactory growth without the fungus but Raynor criticizes this contribution.

In order, therefore, to establish the validity of the claim as to the necessity of the fungus for development of the seedlings, preliminary experiments were begun to note the germination and development of *Calluna*. Various experiments were made, some resulting in failure because of contamination, but finally methods were developed for germinating the seed in the absence of microorganisms. The nutrient solution was prepared according to Raynor's formula. The reaction of the culture medium was varied and in several series of experiments glucose was added to the culture medium. Under strictly aseptic conditions the root system developed normally and with a corresponding good development of the stem. The roots in the better cultures were long, glistening white in color and altogether of healthy appearance. These experiments demonstrate that the fungus is not necessary for proper development of the seedlings and that the obligate relationship emphasized by Raynor is without proof. The fact that the seedlings showed fine root development without the fungus in my experiments whereas in Raynor's experiments the roots were very stubby leads to the suggestion that in Raynor's nutrient solutions some toxic constituent was present. Since Raynor does not give the quantity of iron used but supplied it as a "trace," it is probable that too much iron was used and the iron was then the toxic factor. The growth of the fungus probably changed the reaction of the culture medium to a higher pH value with a consequent precipitation of iron.

Hastening the Germination of Southern Pine Seeds. *Lela V. Barton, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—Stratification effects on longleaf, shortleaf, slash, and loblolly pine seeds were studied in an effort to obtain prompt and complete germination. Growth records showed that stratification for one or two months at 0°, 5°, 10°, or 15° C., or for one, two, three, or four months at 0°, or 5° C. (except longleaf) is of decided benefit in hastening germination and giving a perfect seedling stand. For practical purposes stratification for two months at 5° C. seems desirable except for longleaf which should be stratified for one month at 5° C. or for one or two months at 0° C. These experiments are being extended to include seeds of a number of different species of pine. The results obtained to date indicate that some of these forms respond to stratification while others do not.

Storage, After-ripening, and Germination of Apple Seeds. *William Crocker, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—It has been previously claimed that apple seeds are completely killed by a year of dry storage. Experiments to be reported show that seeds of *Pyrus baccata*, *Pyrus baccata* hybrid, Patten greening, and Wealthy germinate almost equally well after one-half, one and one-half, and two and one-half years of dry storage, provided they are properly after-ripened. After-

ripening requires about two to two and one-half months of moist stratification at a low temperature (0° – 10° C.). The after-ripening proceeds with about equal speed in a considerable range of acidity: granulated peat (pH 3.5–4.5), leached granulated peat (pH 6.–6.5), and neutralized granulated peat (pH 7.–7.2). Contrary to the common belief, a period of dry storage does not lengthen the period of low temperature stratification required. Fully after-ripened apple seeds will germinate even at 0° C., so the period of stratification can not be prolonged much over two and one-half months without loss from excessive germination in the stratification medium. In the fall, hybridizers and nurserymen should thoroughly clean and wash their apple seeds and store, well dried, up to two and one-half months before planting time; then stratify in a moist medium at low temperature and plant at the end of two months.

Types of Root Growth from Cuttings with Special Reference to Position on the Stem. *P. W. Zimmerman and A. E. Hitchcock, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—From external appearances, origin of roots from stem cuttings of various species of plants falls into several categories, as follows: (1) from the bark just above the basal cut end, thus indicating strong polarity (*Ligustrum japonicum*); (2) distributed around the node (*Viburnum Opulus*); (3) from the axil of the bud, appearing to be related to the bud gap (gooseberry); (4) from the callus, showing relationship to tissues formed after the cutting was made (*Picea pungens*); (5) one root immediately above and one immediately below the node (*Lycium* sp.); (6) arising promiscuously about the stem without any apparent relationship to particular structures of the bark (*Viburnum tomentosum*); (7) rows of roots running parallel to the stem as if related to medullary rays (*Evonymus japonicus*); (8) at nodes in particular, and internodes in general (currant); (9) from basal part of new shoots as they arise from the old stem (Dorothy Perkins rose). Most cuttings show basal rooting even though they fall in one of the other categories mentioned above. Very young cuttings usually root first from the basal part, though as the wood hardens they show strong local tendencies. The practice of cutting "to the node" is good for some species, but has no special significance in the majority of cases.

The Influence of Leaves and Buds on the Type of Roots Developed by Cuttings. *P. W. Zimmerman and A. E. Hitchcock, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—Van der Lek found that the presence of buds on grape cuttings influenced the position and type of roots which developed. He formulated the theory that special substances (hormones) determined the development of roots. In experiments conducted by the writers to determine how light and the presence or absence of buds affect the type of roots formed by cuttings, the following results were ob-

tained for several species of plants: (1) in light, cuttings with leaves produced many main roots having a large number of secondaries; (2) budless cuttings produced fleshy roots with few or no secondaries; (3) cuttings kept in the dark (buds not removed) produced normal main roots having few secondaries; (4) in light, cuttings which had their leaves removed as they appeared, produced roots similar to those of cuttings kept in the dark. Microchemical tests gave results as follows: at the beginning the cuttings contained an abundance of starch in the pith and bark and a plentiful supply of sugar throughout; at the end of the experiments leafy cuttings contained less starch in the old wood than was found in budless cuttings; starch was found only in the endodermis of the stems of new shoots; cuttings with leaves removed as they first appeared and cuttings kept in the dark were low in starch and sugar; there is some indication that the leafy shoot in light is responsible for the type of root system developed; there seemed to be no correlation between the type of roots and the carbohydrate supply.

Water Intake by Cuttings. *P. W. Zimmerman and A. E. Hitchcock, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—It has been supposed that water must pass into cuttings through the cut end of the stem. The results of several experiments indicated that entrance might be gained in other ways; therefore the writers have tried to determine whether water can be taken in through bark, leaf scars, and leaves. Cut ends of leafy stems were sealed with paraffin and immersed in water in graduated cylinders for comparison with untreated stems. The results indicate that species vary greatly in their capacity to absorb water. Some kinds of plants are dependent upon the cut end of the stem for water absorption, while others remain turgid when the cut end is sealed. Injured places where petioles had been removed often acted as important points for water intake. When both scars and cut end are sealed wilting usually results. Sealed cuttings of some species that wilt quickly in dry air can be revived if placed in high humidity, thus indicating slightly permeable bark. Permeability of leaves varied as did stems. A few species remain turgid if the tips only are immersed in water, but most types wilt with such treatment.

Physiological Advantages of the Nurse-graft Y-Cutting Method of Propagating Plants. *Walter T. Swingle, T. Ralph Robinson, and Eugene May, Jr., U. S. Dept. Agr., Washington, D. C.*—Plants extremely difficult to root from cuttings strike root readily when propagated by this new method described in a recent number of the *Journal of Heredity*. There are physiological advantages of this method of rooting cuttings over any previously used. Water absorbed by the roots containing dissolved mineral salts will, if necessary, move backward through the wood of leafy branches, and it will move upward through a part of a tree trunk that had been killed by prolonged exposure to steam. There is evidence that carbo-

hydrates and nitrogenous reserve plant foods move downward in the phloem elements of the cortex and do not move or at least move with great difficulty in the reverse direction. When the Y-cutting is placed in the propagating bed with a well rooted nurse-plant grafted to one of its arms, the soil solutions absorbed by the roots of the nurse-plant circulate freely to both arms of the Y-cutting, enabling the leaves of both branches to synthesize both carbohydrate and nitrogenous plant foods. The transportation of such reserve plant foods in the direction of the root will bring all of such reserve foods originating in the ungrafted arm of the cutting (and much of that produced in the grafted arm) to the base of the Y-cutting where roots are forming. This is because such reserve food materials after moving down one branch of the Y-cutting to the crotch cannot move up the other arm to the union with the nurse-graft, but must go on down to the base of the cutting. A trap action is in operation, by virtue of which reserve plant foods accumulate rapidly at the base of the cutting just when roots are forming and growing.

Effect of Chemicals, Temperature, and Humidity on the Lasting Qualities of Cut Flowers. *A. E. Hitchcock and P. W. Zimmerman, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—In view of the fact that aspirin has been in general use as a means of prolonging the life of cut flowers, this compound and 43 other chemicals were tested with several species of flowers. Among the other chemicals used were inorganic and organic acids, alkaloids, alcohols, and inorganic and organic salts. None of these chemicals increased the life duration of the flowers beyond that of the checks. Low temperature (3° to 10° C.) was the most effective means of keeping cut flowers fresh over a long period of time. Fresh cut flowers lasted longer in the laboratory than those that were removed from cold storage. Various relative humidities were obtained in bell jars where the flowers were kept, by first passing air through a series of flasks containing known concentrations of sulphuric acid and water. A low humidity at room temperature was more favorable for some species than a high humidity. Selection of comparable flowers was based on their age after opening from the bud stage. This procedure is considered of particular importance since it precludes marked variations in response of individual flowers picked at random.

Further Studies on Malol from Apple Peels, Prunol from Wild Cherry Leaves, and Urson from Bearberry Leaves. *Charles E. Sando, U. S. Dept. Agr., Washington, D. C.*—Malol, prunol, and urson are identical, but the formula is $C_{30}H_{48}O_3$ and not $C_{31}H_{50}O_3$ as suggested by van der Haar. This conclusion is supported by forty combustions of the parent substance and further combustions of the following derivatives: diacetyl derivative, monoacetyl derivative, monomethyl derivative, acetylmethyl derivative,

regenerated methyl derivative, phenacyl derivative, phthalic acid ester, and phthalic acid ester of methyl derivative. Further evidence of the presence of a COOH group is given by chlorinating the monoacetyl derivative with thionyl chlorid, boiling with methyl alcohol, and reacylating—thus obtaining the monoacetyl-monomethyl derivative which appears to be identical with the same compound prepared by methylating the parent substance with sodium ethoxid and methyl iodid and acetylating this product. The formation of the acetylmethyl derivative by the use of thionyl chlorid is probably only possible by assuming the presence of a COOH group. Van der Haar makes the statement that the presence of a COOH group precludes the possibility of a diacetyl derivative being formed. This is refuted. No difficulty is experienced in preparing such a derivative which proves to be probably the monoacetyl derivative of the mixed anhydrid.

The Effect of Spiral Ringing on Solute Translocation and the Structure of Regenerated Tissues. *L. H. MacDaniels and O. F. Curtis, Cornell University, Ithaca, N. Y.*—To test out the efficacy of lateral conduction in the phloem, apple trees 3 to 5 inches in diameter were ringed by removing a strip of conducting tissue extending spirally twice around the trunk from left to right upward. In some cases this strip was composed of bark only, in others of both bark and an annual layer of wood. The vertical distances between the ends of the spiral were 6, 9, 12, and 18 inches on different trees. Nitrogen analyses of the leaves indicated that that part of each tree immediately to the left of the upper end of the spiral was similar to the completely ringed tree and had received little or no nitrogen, whereas in the part to the right of the end of the spiral, nitrogen had increased about as in the check trees. This showed that the upward moving nutrients did not move laterally after having passed around the spiral. During the second season the localized effects of nitrogen starvation disappeared, probably due to readjustment in the conducting tissues. There was a high positive correlation between nitrogen content and high catalase activity of the leaf tissues. Downward movement of solutes was hindered by the spiral ring as indicated by increased fruit-bud formation and fruiting on the spirally ringed trees. (Such movement was not wholly prevented however, as was shown by growth along the entire length of the spiral and by diameter increase on the trunk below.) Anatomical examination showed that new tissues had been laid down along the line of the spiral on the upper side. The conducting elements of this new tissue were parallel to the spiral, the change in their orientation being due to a change in the orientation of the cambium. Lenticels formed on the regenerated bark showed this change in orientation also.

A Preliminary Study of the Diffusion of Sugars through Membranes of Living Plant Tissue. *F. C. Steward, Cornell University, Ithaca, N. Y.*—

The work was undertaken in order to ascertain how solutes traverse a region of living, unspecialized, parenchyma cells. Special significance has recently been ascribed to the view that the solutes in such cases diffuse along the cell walls rather than across protoplasts, and the utility of this idea in explaining certain facts of growth and development in the plant has been indicated by J. H. Priestley. A simple and convenient technique for mounting membranes composed of living plant tissue for such study was devised, in which cut slices of storage organs (potato, beetroot) and discs of bark from cherry and maple trees were mounted in such a manner as to reduce to a minimum the possibility that the diffusing sugar was carried by vascular tissues. It was found that the amount of diffusion of sugars with relatively steep concentration gradients was low. This is attributed primarily to the relative impermeability of the protoplasm of these living cells to sugars. Pumping out the intercellular air under water or withdrawal of the protoplasm from the cell wall by plasmolysis, thereby increasing the space between protoplasts available for diffusion, increases the rate. Permeability changes induced by various treatments are of relatively slight importance. The rate of longitudinal diffusion of glucose in bark with a definite concentration gradient was ascertained. It is concluded that the protoplasm of living cells resists the passage of sugars by simple diffusion. The small amount of diffusion actually obtained with unplasmolyzed cells may be due to slight permeability of the protoplasm or diffusion along cell walls or intercellular spaces flooded with water. Whether this is of sufficient magnitude to account for the passage of solutes across such regions in the living plant is a matter for some discussion. It is tentatively suggested that some mechanism is present in the plant which facilitates diffusion across unspecialized cells. In this connection the suggestion of Curtis that the streaming protoplasm hastens diffusion in the phloem is of interest.

Studies Indicating That Translocation is Dependent on the Activity of Living Cells and the Possible Bearing of This on Solute Distribution. *Otis F. Curtis, Cornell University, Ithaca, N. Y.*—The temperature of a leaf petiole or that of a stem may be controlled at will by encasing it in a coil of small rubber tubing through which water at the desired temperature is passed. When the temperature of the conducting tissue is thus lowered to somewhere between 1° and 6° C., removal of carbohydrate from the leaf or part above the chilled region and the movement of inorganic salts into these parts from below are stopped or very much reduced. The fact that the temperatures necessary to cause a check in translocation of solutes approximate those that cause a cessation of protoplasmic streaming supports the previous suggestion that protoplasmic movements in the conducting cells play a necessary part in rapid translocation. Excluding oxygen from the petioles by passing nitrogen through an enclosing cylinder of copper foil has been found to interfere with sugar removal from the leaf blade. This

evidence also indicates that active living cells are necessary for normal translocation. The evidence that streaming protoplasm is necessary for rapid transfer of solutes suggests that an important factor determining the partition of solutes between competing organs, such as buds or growing fruits, and the rapid movement of foods into or out of storage tissues may be that factor which initiates or maintains a continuous chain of living cells having active protoplasmic movement and connecting that tissue using or storing the solutes with the tissue supplying them. The rapid filling or emptying of storage organs when attached to normal living tissues as contrasted with the very slow filling or emptying that has been observed when placed in solutions or in water may be accounted for by the presence in the former case of some factor or factors inducing protoplasmic movement. Some such factor initiating or maintaining protoplasmic movement may be of greater importance in determining normal absorption or loss of solutes than are changes in permeability.

The Translocation of Water in Citrus Fruits. *H. S. Reed, Citrus Exp. Sta., Riverside, Calif.*—The water-transporting system of citrus fruits differs in many respects from that of other fruits. The fibrovascular system is richly developed in the mesocarp and very slightly developed in the endocarp. Fluids move by diffusion from the mesocarp to the endocarp, especially to the juice sacs in the locules. The rate of swelling in various solutions shows that the pectose layers of the mesocarp cells play an important rôle in translocation. Poisonous solutions induce a distention of the fruits as readily as equimolecular solutions of other salts, but ions which combine with pectose compounds alter the rate of swelling. At a maintained temperature of 25° to 26° C. most of the swelling occurs in the first eight hours; later the fruits may shrink. The wall of the juice sac consists of modified cellulose which is not readily permeable to dyes or salts.

A Study of the Stimulating Effect of Various Fertilizers on the Sugar Metabolism of the Plant. *G. C. Wickwire and W. E. Burge, University of Illinois, Urbana, Ill.*—The object of this investigation was to determine the effect of fertilizers on sugar metabolism. The respiratory quotient is the index usually used to the amount of sugar metabolized. In this investigation sugar utilization, as well as the effect of the fertilizers on this utilization, was determined directly according to the following procedure. A large quantity of *Spirogyra porticalis* of almost pure culture was collected and the excess water was removed by gently squeezing with the hands. This large batch of *Spirogyra* was divided into several portions of 40 grams each. Each of the 40-gram portions was introduced into 200 cc. of 0.1-percent dextrose solution in flat-bottom dishes 8 cm. in diameter. 200 mg. of each of the fertilizers, "vigoro," "bone meal," "mixed chemical," "succo," "nitrate of soda," "dried blood," and "Armour's Lawn," were added to the

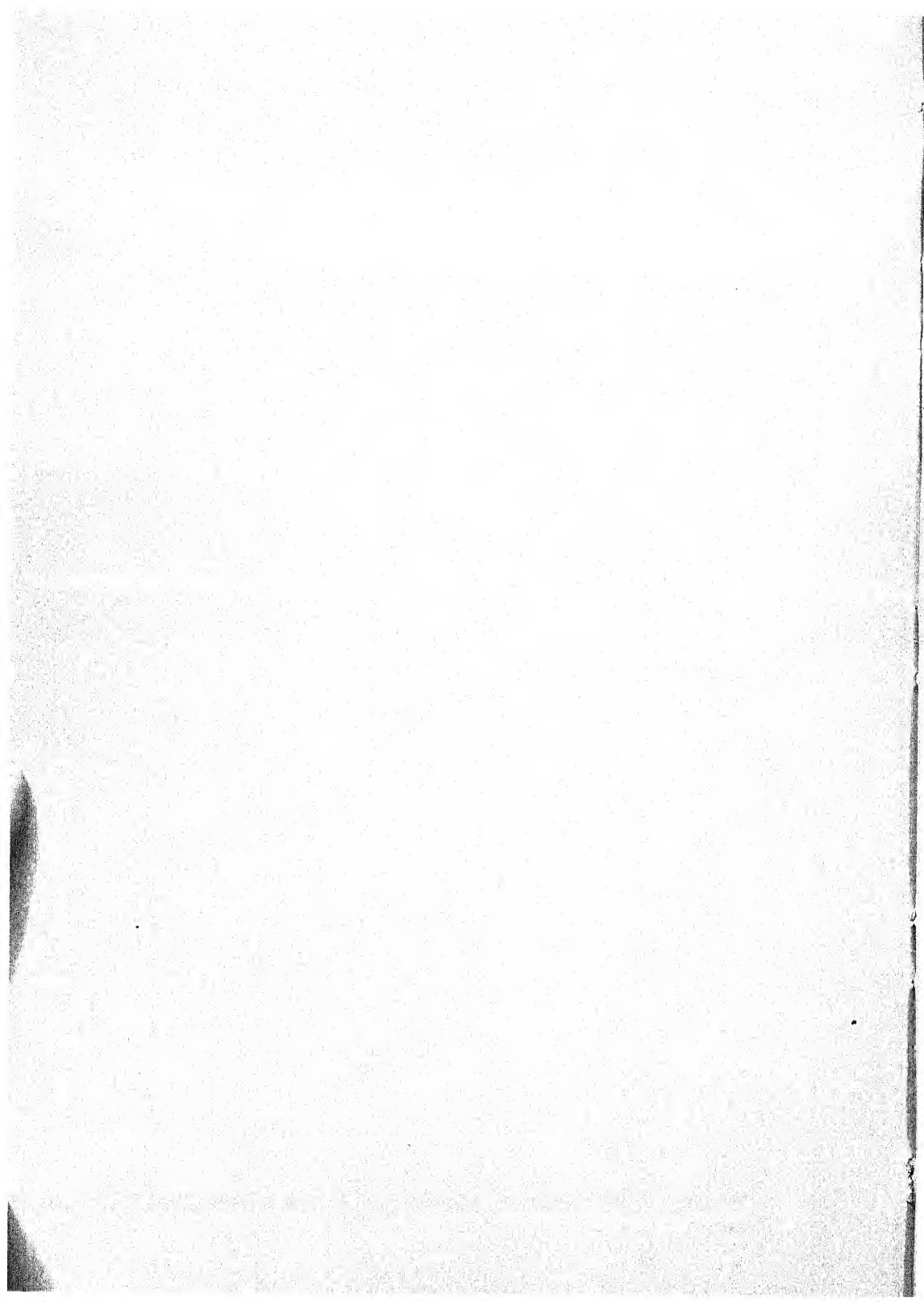
Spirogyra sugar preparations. Preparations to which no fertilizers were added served for controls. 15 cc. of liquid were removed from each of the dishes and sugar determinations were made. The preparations were then permitted to stand at ordinary room temperature for 30 hours and sugar determinations were again made. Following are the average figures for four sets of experiments: the control used 15 percent of the sugar in 30 hours; the *Spirogyra* sugar preparation to which "vigoro" was added, 25 percent; that to which "bone meal" was added, 47 percent; "mixed chemical," 60 percent; "succo," 50 percent; "nitrate of soda," 58 percent; "dried blood," 45 percent; and that to which "Armour's Lawn" was added, 52 percent. By comparing these figures it will be seen that all of the fertilizers increased sugar utilization above the control, and that "mixed chemical" produced the greatest increase.

Some Studies on Sugar Extraction from Beets, and Sugar Distribution in Beets. *Ernest Reed, Syracuse University, Syracuse, N. Y.*—The cold water extraction method in which the pulp is secured from the beet by a mechanical device, such as a drill, is found to be unsatisfactory. A new method is explained. This method includes the use of hot water and pressure with very satisfactory results. The distribution of the sugar in the beets was studied by means of testing tubes of the pulp taken from all parts of the beet; the sugar distribution was found to be uniform throughout the beet.

The Effect of Temperature and Light upon the Development of Corn Endosperm. *Lois Lampe, Ohio State University, Columbus, Ohio.*—At the Philadelphia meeting of this section the results of a morphological and microchemical study of developing corn endosperm were reported. At that time it was shown that both the development of cells and the consequent distribution of food materials in the endosperm are correlated with regional activity which gives rise to gradients in the growing endosperm. The present report deals with the duration of the gradients as affected by the temperature and light conditions prevailing during the time the kernels are growing. Summations of temperature expressed in hour-degrees Fahrenheit failed to show any correlation with the rate of development of the endosperm. In serial plantings the intensity of the temperature above the point critical to growth, and the duration of such intensity correlate closely with the rate of development. Appleman found that the rate of ripening of the corn kernel under mid-summer field conditions is in accord with the Vant' Hoff-Arrhenius principle. Serial plantings in this experiment show that the principle holds for the range of temperature between mid-summer temperatures and the minimal critical temperature. Summations of light increments, expressed in gram-calories, received by the kernels during the time they were growing, also failed to show any correlation with

the rate of development of the endosperm. The photosynthetic activity of the plants previous to pollination and afterward furnished an adequate food supply, and any other effects of light under the conditions of the experiment were hidden by the greater response of the developing kernels to the wide variation in temperature.

The Rôle of the Mother Tuber in the Growth of the Potato Plant. *F. E. Denny, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—To determine at what period of growth the young potato plant becomes independent of the stored food in the mother tuber, and to study the chemical changes occurring in the mother tuber during the transference of materials from the seed piece to the sprout, amputation experiments were carried out in 1926, 1927, and 1928. The method of amputation permitted the removal of the mother tuber and the replacement of the plant in the soil without disturbance of the root system. The subsequent rate of growth and final yield of amputated plants were compared with those obtained from check plants subjected to the same procedure except that the mother tubers were not removed. Amputation of mother tubers at emergence of sprout or when the young plant was two inches high reduced the yield. Removal of mother tubers when plants were 10 inches high resulted in a slight decrease for Irish Cobbler, but with Bliss Triumph the yield from plants allowed to retain their mother tubers was less than that from the amputated plants. The cause of this behavior is discussed. Chemical analyses of the tissue amputated at each stage of development are given in detail. About 70 to 85 percent of the dry weight of the seed piece was utilized, all forms of substances decreasing in amount in the seed piece during growth except sugar and water, both of which increased markedly.



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